A STUDY OF CULTURES FROM SPUTUM AND BLOOD IN LOBAR PNEUMONIA.*

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The researches of Weichselbaum proved that the pneumococcus was the etiological factor in the majority of the cases of lobar pneumonia which he investigated, and those cases in which the organism of Welch and Fraenkel could not be isolated were therefore considered of minor import. In this latter class of cases Friedländer's bacillus was usually thought to be the causative agent. Pearce (1) isolated the pneumococcus in all but three of the 125 cases that he reported (97 per cent.), and he believes that in these three it was present at some time in the course of the disease.

In 1905 Schottmüller (2) assigned a prominent rôle to *Streptococcus mucosus* as an etiological factor, and yet since that date lobar pneumonias of origin other than the pneumococcus have been but little considered. Lyall (3) in 1912 reported a series of forty-two cases of pneumonia in three of which he isolated a streptococcus from the blood.

From the widely varying results in the bacteriological examination of patients having pneumonia, the impression is created that some factors other than differences in technique must be responsible for the differences in the results. From the results of work carried on at the Laboratory of Clinical Pathology at Cornell, we concluded that the widely varying results are due to the fact that lobar pneumonia may be caused by a variety of organisms.

Since 1903 a yearly study of the sputa in respiratory tract infections has been made from patients of the Cornell Dispensary and the Second Division wards of Bellevue Hospital. A report of these cases by Hastings and Niles (4) includes sixty-six cases of lobar pneumonia, in forty-three of which the pneumococcus was not

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found. This study led to the conclusion that "lobar pneumonia may produce sputum free from pneumococci, and may undoubtedly be caused by organisms other than the pneumococcus." The present paper is a continuation of that report and deals with some cases of lobar pneumonia from the Second Division of Bellevue Hospital during 1911. The work, however, is amplified in that the sputum studies were controlled by blood cultures from most of the cases.

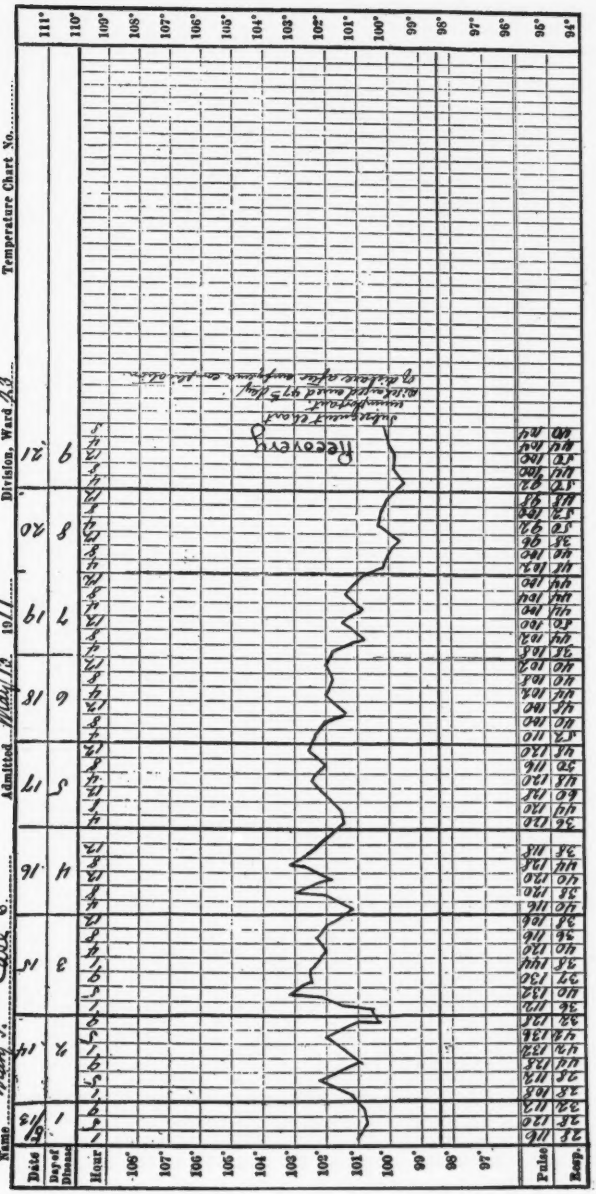
Niles and Meara (5) in 1911 reported two cases of lobar pneumonia, not caused by the pneumococcus, which are included in this series.

In one case *Micrococcus catarrhalis* was isolated several times from the sputum, and one blood culture from this case gave a negative result. The case was admitted to the hospital and is described as being "evidently primarily an arthritis." The conclusion of Meara and Niles, that "it seems much more logical to attribute all of the phenomena to an acute pyogenic infection, which in this instance it would seem fair to assume was the *Micrococcus catarrhalis,*" is unwarranted. *Micrococcus catarrhalis* has never been isolated from the blood, and has never been known to cause a general pyogenic infection.

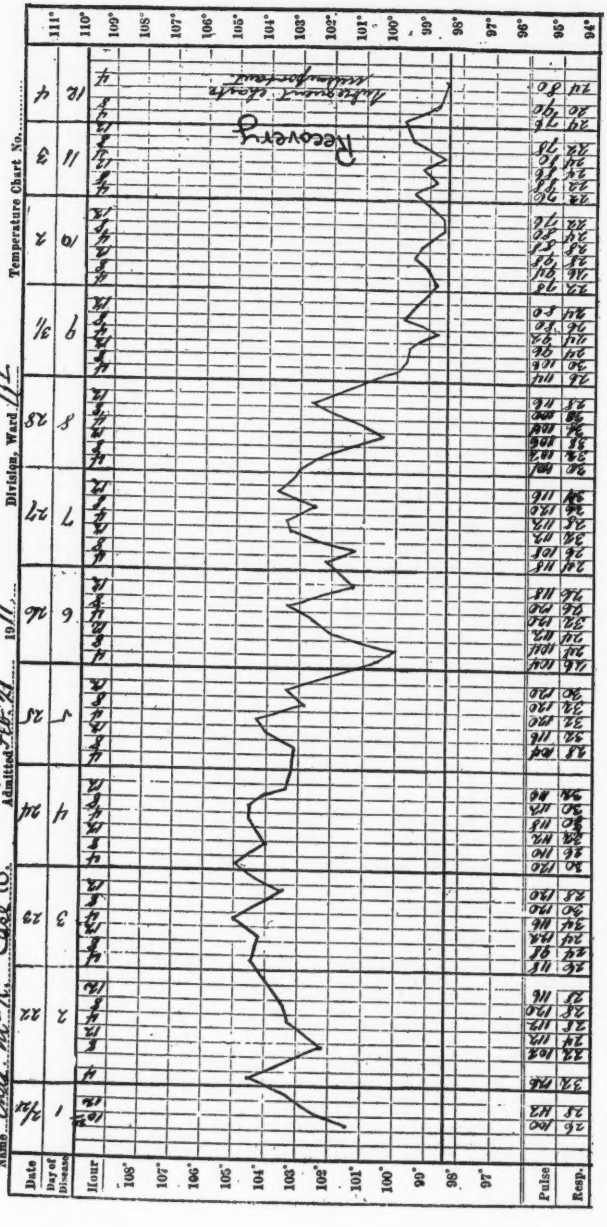
In the second case Meara and Niles reported obtaining *Bacillus coli communis* in pure culture from the sputum upon two examinations, and at the same time two negative blood cultures. These two cases are among the few reported in the literature in which cultures from the blood and from the sputum were taken at approximately the same time during the course of lobar pneumonia.

The present report comprises thirty-two cases in which blood cultures and sputum cultures were taken. A growth occurred in eleven of the blood cultures (34 per cent.). Of the eleven positive cases, the pneumococcus was found in nine and *Streptococcus hemolysans* in two. In all the cases in which the pneumococcus was isolated from the blood, it was isolated also from the sputum, and in the two cases showing streptococcemia the streptococcus was isolated from the sputum.

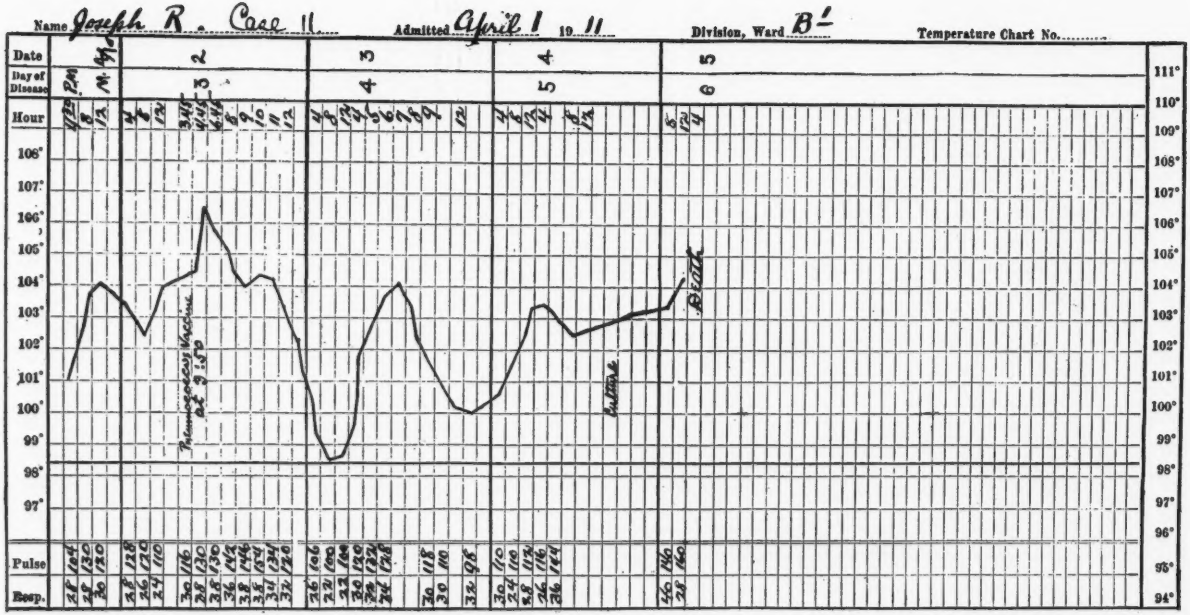
For the blood cultures the usual routine was followed. The blood was drawn into sterile Ewing tubes, and immediately poured



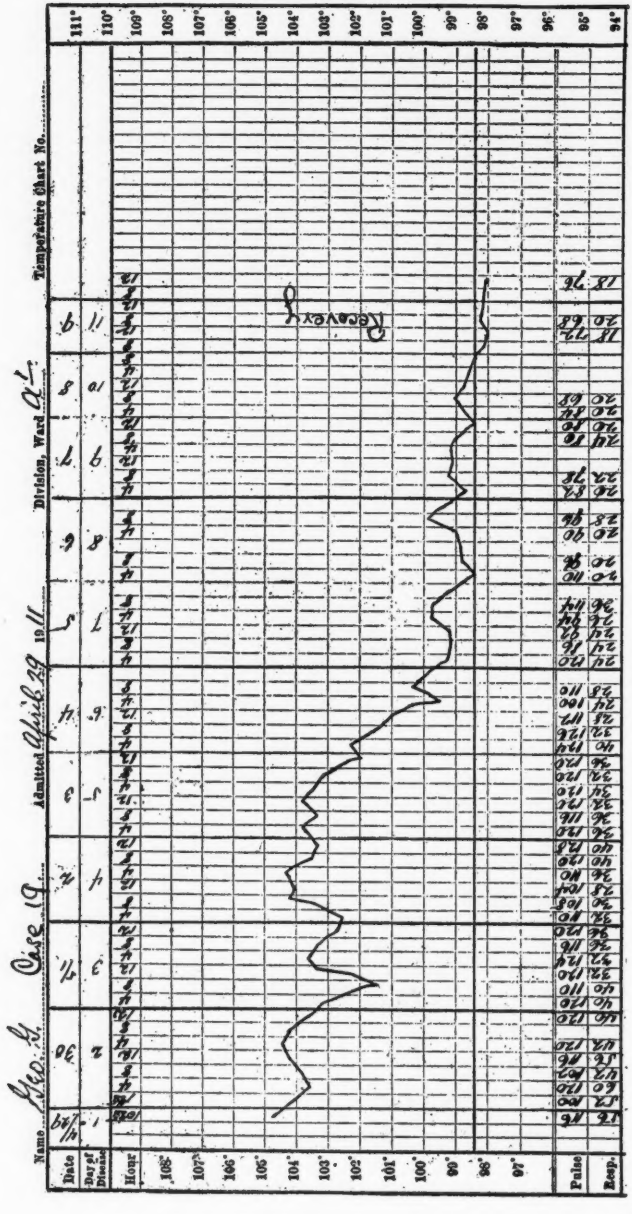
TEXT-FIG. 1. From a typical case with pneumococci in both the blood and the sputum.



TEXT-FIG 2. From a streptococcus case. The history, physical examination, and course of the case were typical of lobar pneumonia as it is ordinarily seen. *Streptococcus hemolysans* was found in the blood and the sputum.



TEXT-FIG. 3. From a streptococcus case. The history, physical examination, and course of the case were typical of lobar pneumonia as it is ordinarily seen. *Streptococcus hemolysans* was found in the blood and the sputum.



TEXT-FIG. 4. From a typical case with pneumococci in the sputum. The blood was negative.

TABLE I.

Case No.	Blood culture.			Sputum culture.	Remarks.
	Medium.	Dilution.	Result.		
1	Bouillon	1:50	+	Pneumococcus	Fatal case.
		1:75	+Pneumococcus		
	Agar plates		+		
2	Bouillon	1:25	+	Pneumococcus	
		1:50	+Pneumococcus		
		1:75	+		Fatal case.
	Agar plates		+		
3	Bouillon	1:25	+Pneumococcus	Pneumococcus	Fatal case.
		1:75	+		
4	Bouillon	1:25	+	Pneumococcus	Recovered.
		1:50	+Pneumococcus		
	North's medium plates		+		
5	Bouillon	1:25	+	Pneumococcus	Fatal case.
		1:50	+Pneumococcus		
	Agar plates		+		
6	Bouillon	1:25	+	Pneumococcus	Sputum not rusty. Patient recovered (text-figure 1).
		1:50	+		
		1:75	+Pneumococcus		
	Agar plates		+		
7	Bouillon	1:25	+	Pneumococcus	Fatal case.
		1:50	+Pneumococcus		
	North's medium plates		+		
8	Bouillon		+Pneumococcus	Pneumococcus	Recovered.
	Agar		+		
9	Bouillon		+Pneumococcus	Pneumococcus	Recovered.
	Agar		+		
10	Bouillon	1:50	+	*Streptococcus hemolysans (longus)*	*S. hemolysans (longus)* also in blood. Patient syphilitic. Typical lobar pneumonia clinically. Recovered (text-figure 2).
		1:75	+Streptococcus		
	Agar plates		+		

Case No.	Blood culture.			Sputum culture.	Remarks.
	Medium.	Dilution.	Result.		
11	Bouillon	1:25	+	*Streptococcus hemolysans (longus)*	Blood cultures and sputum both showed the streptococcus. Sputum rusty and viscid. Patient died (text-figure 3).
		1:50	+		
		1:75	+Streptococcus		
	North's medium plates		+		
12	Bouillon	1:50	−		Two blood cultures negative. Pneumococci in sputum both times. Fatal case.
		1:75	−	Pneumococcus	
	Agar plates		−		
13	Bouillon	1:25	−		Subsequent empyema. Three blood cultures negative. Recovered.
		1:50	−	Pneumococcus	
		1:75	−		
	Agar plates		−		
14	Bouillon	1:25	−		Patient alcoholic. Recovered.
		1:50	−	Pneumococcus	
	Agar plates		−		
	North's medium plates		−		
15	Bouillon	1:25	−		Massive pneumonia. Recovered.
		1:50	−		
		1:75	−	Pneumococcus	
	Agar plates		−		
	North's medium plates		−		
16	Bouillon	1:50	−		Sputum not blood-tinged. Recovered.
		1:75	−	Pneumococcus	
	North's medium plates		−		
17	Bouillon	1:25	−		Alcoholic. Fatal case.
		1:50	−	Pneumococcus	
	Agar plates		−		
18	Bouillon	1:25	−		Recovered.
		1:50	−	Pneumococcus	
	Agar plates		−		
19	Bouillon	1:25	−		Recovered (text-figure 4).
		1:50	−	Pneumococcus	
	North's medium plates		−		
20	Bouillon		−	Pneumococcus	Recovered.
	Agar		−		
21	Bouillon	1:50	−	*Bacillus coli communis*	Subsequent suppurative pleurisy. *B. coli communis* in exudate. Fatal case.
		1:75	−		
	Agar plates		−		

Case No.	Blood culture.			Sputum culture.	Remarks.
	Medium.	Dilution.	Result.		
22	Bouillon		—	*Micrococcus catarrhalis*	Acute arthritis. Tonsillitis. Lobar pneumonia. Recovered. One culture.
	Glycerin agar		—		
	Plain agar		—		
23	Bouillon		—	*Bacillus coli communis*	Two blood cultures negative. Recovered.
	Glycerin agar		—		
	Plain agar		—		
24	Bouillon		—	Streptococcus and staphylococcus	Recovered.
	Agar		—		
25	Bouillon	1:50	—	No pneumococcus	Recovered. Many staphylococci in sputum.
		1:75	—		
	Agar plates		—		
26	Bouillon	1:25	—		Patient quite toxic. Unidentified Gram positive chromogenic organism predominant in sputum. Recovered.
		1:50	—	No pneumococcus	
		1:75	—		
	Agar plates		—		
27	Bouillon	1:25	—	No pneumococcus	Few streptococci in sputum with many staphylococci. Patient alcoholic. Recovered.
		1:50	—		
	North's medium plates		—		
28	Bouillon	1:20	—		*B. influenzæ* isolated. Patient alcoholic. Recovered.
		1:50	—	No pneumococcus	
	Agar plates		—		
29	Bouillon	1:25	—	No pneumococcus	Subsequent empyema. Unidentified Gram negative bacillus in sputum. Recovered.
		1:50	—		
	Agar plates		—		
30	Bouillon	1:25	—		
		1:50	—	No pneumococcus	
		1:75	—		Fatal case.
	Agar plates		—		
31	Bouillon	1:25	—		Very toxic and delirious. Developed acute mastoiditis. Recovered. Two blood cultures, both negative.
		1:50	—	No pneumococcus	
		1:75	—		
	Agar plates		—		
32	Bouillon	1:25	—		
		1:50	—	No pneumococcus	Recovered.
	North's medium plates		—		

into flasks of broth in dilutions of 1:25 to 1:75. Blood agar plates were also made in varying dilutions, 1:3 to 1:12. No special media, such as that suggested by Wiens (6), were used, as in our experience growth took place as readily on the ordinary as on the special media. Strouse and Clough (7) in their reported series of cases seem to have had a similar experience. In all our positive cases growth occurred in both the liquid and solid media.

The sputa reported in this series were clean specimens, *i. e.,* free from varied bacterial and fungoid growth, buccal cells, and foreign particles. This is easily determined from a smear. The sputa were collected and washed after the method of Kitasato (8). After being washed, the selected portion of sputum was streaked upon plates of North's medium and upon blood agar, and from these colonies were later transferred to suitable media for differentiation. Three smears from each sputum were made. One was stained with methylene-blue, and showed at a glance whether the specimens were clean. The second was stained by Gram's method, and the third by Hiss's method for capsules. In no case were pneumococci recovered in culture where they were not seen in the smear preparations; and conversely, they were always procured in culture when present in smears.

The pneumococci and different streptococci isolated were referred to the laboratory for further study and for the purposes of this study were classified according to Schottmüller, except for slight modifications. The variations noted by those who have made particular studies of similar organisms were also investigated. The classification is as follows:

Streptococcus pyogenes (*longus et erysipelatis*).
Streptococcus hemolysans (*longus sue brevis,* Schottmüller).
Streptococcus mitior (*viridans, tenuis*) (*longus sue brevis,* Schottmüller).
Pneumococcus (Welch and Fraenkel).
Streptococcus mucosus (Perkins and Howard, and Schottmüller).
Micrococcus zymogenes (Hastings and Macallum).
Enterococcus (Thiercelin),—probably a *Streptococcus mucosus.*

In addition to the usual blood, glucose, and special media, such as North's and nutrose media, the following carbohydrate media were utilized.

Monosaccharids
Dextrose, Levulose, Galactose } Hexoses

Disaccharids
- Saccharose
- Lactose
- Maltose

Polysaccharids
- Dextrin
- Inulin

Alcohols
- Mannite
- Dulcite

Our blood cultures show a low percentage (30.3 per cent.) of positive results for pneumococcus, which is in harmony with the results of Schottmüller, who obtained 23 per cent. of positive cultures out of 209 cases, and with those of Cole (9), who obtained 30 per cent. of positive cultures from thirty cases. These figures differ widely from those of other workers, notably Prochaska (10), who reported 100 per cent. of positive cultures out of forty cases, Rosenow (11), who obtained 91 per cent. out of 145 cases, and Kinsey (12), who reported 76 per cent. positive out of 25 cases. Kinsey seemed to think that the degree of dilution of the blood was a most important factor. We did not think that the dilution was so important, and our own work and that of others (Strouse and Clough (7)), who mention their dilutions, confirmed our opinion.

Our cases are summarized in table I.

SUMMARY.

In the thirty-two unselected cases studied, cultures were taken from both the blood and the sputum, and in five of these more than one blood culture was taken.

In one case the culture was taken from the blood only, and in this instance the pneumococcus was found. This case will be considered only with reference to the incidence of the pneumococci in blood cultures in lobar pneumonia.

The results in the thirty-two cases in which cultures were made both from the blood and the sputum are as follows:

1. In eleven cases the blood and sputum cultures were positive.

(*a*) In nine cases the pneumococcus was isolated, and five of these patients died.

(*b*) In two cases, instead of the pneumococcus, *Streptococcus hemolysans* (*longus*) was found. One of these patients died.

2. In twenty-one cases the blood cultures were negative, and in eighteen of these the sputum cultures were positive and in three negative. In these three the only result recorded was the absence of the pneumococcus.

(*a*) In nine cases the pneumococcus was isolated. Two of these patients died.

(*b*) In twelve cases no pneumococcus was found. Two of these patients died.

Of the patients in which no pneumococcus was found, in two cases *Bacillus coli* was isolated; in one case *Micrococcus catarrhalis;* in one case a staphylococcus (type not stated); in two cases staphylococcus and streptococcus; in one case *Bacillus influenzæ;* in one case *Bacillus fluorescens non-liquefaciens;* in one case an unidentified Gram positive chromogenic organism, probably saprophytic; and in three cases there are no recorded results except that the pneumococcus was absent.

In the total of thirty-three blood cultures, the pneumococcus was found in ten cases (30.3 per cent.), and six of the patients died. During the years 1910 to 1912 sputum cultures were taken from forty-four cases of pneumonia, of which twenty-four showed pneumococci (54 per cent.), and twenty showed no pneumococci (45 per cent.).

CONCLUSIONS.

1. Typical cases of lobar pneumonia, in which no pneumococci are to be found either in the blood or sputum, probably exist more frequently than is generally supposed.

2. These cases are not to be distinguished from the pneumococcus group except by blood and sputum cultures.

3. *Streptococcus hemolysans* (*longus*) is an organism which may give rise to such a case. In this connection it is of interest to note that Schottmüller (13) isolated *Streptococcus mucosus capsulatus* from the blood in a case of croupous pneumonia.

There is probably no prognostic value in the bacteriological findings in lobar pneumonia, but from a series of thirty-two cases we believe no definite opinion can be drawn.

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5. Niles, W. L., and Meara, F. S., *Am. Jour. Med. Sc.*, 1911, cxlii, 803.
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PANCREATIC TRANSPLANTATIONS IN THE SPLEEN.*

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Plates 51 and 52.

It is now acknowledged by all authorities that total removal of the pancreas produces diabetes. Minkowski[1] found, however, that the presence of a subcutaneous transplant of a portion of the pancreas would prevent the development of diabetes provided that the blood supply of the graft was left intact.

The lower part of the pancreas in the dog lies free in the mesentery. This part of the gland has been named by Pflüger[2] the processus uncinatus, and it is joined above to the corpus pancreatis which is in close contact with the duodenum. Into the lower end of the processus uncinatus enter good sized vessels, branches of the inferior duodenal artery and vein. Minkowski cut through the processus uncinatus in its upper part. The portion of the pancreas thus freed was drawn out of the abdomen and placed in a prepared pocket in the subcutaneous tissue. The blood vessels entering the tip of the gland were carefully preserved, and formed a vascular stalk which passed from the peritoneal cavity through the incision in the anterior wall of the abdomen to the graft. The external secretion of the gland was discharged through a fistulous opening in the skin. After such a graft had been made the removal of the intra-abdominal remains of the pancreas did not produce persistent glycosuria. Usually the urine contained sugar for twenty-four hours or less after the operation. Subsequent extirpation of the graft by the simple extraperitoneal operation of removing the graft, without injury to any other structures, produced a severe and rapidly fatal

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[1] Minkowski, O., *Berl. klin. Wchnschr.*, 1892, xxix, 90; *Verhandl. d. Cong. f. inn. Med.*, 1892, xi, 189; *Arch. f. exper. Path. u. Pharmakol.*, 1893, xxxi, 118.

[2] Pflüger, E., *Arch. f. d. ges. Physiol.*, 1907, cxviii, 267.

diabetes. Minkowski's experiments were soon repeated and the results were confirmed by Hédon[3] and Thiroloix.[4] This experiment has formed one of the chief supports for the theory that the pancreas produces an internal secretion. It led Thiroloix to abandon his theory that the diabetes that followed extirpation of the pancreas was due to gross traumatic lesions of the nerves or nervous plexuses in the neighborhood, and to adopt the theory of an internal secretion.

Pflüger[5] insisted that the graft experiment did not overthrow the neurogenic hypothesis, and pointed out that the nerve supply of the portion of the pancreas that formed the graft was unchanged, as the nerves that accompanied the vessels in the mesenteric stalk remained intact and held the graft in connection with the nerve centers.

When Minkowski cut this stalk, diabetes resulted although the graft was left. Pflüger explained that this might be due to severing of the nerves rather than to interference with the blood supply. "If centripetal nerves passing from the pancreas can inhibit the activity of the sugar centers of the brain and spinal cord, then the section of these inhibitory nerves would result in an increased production of sugar" (Pflüger).[6]

Hédon in 1892 reported one experiment in which the vascular stalk of the graft was tied without diabetes resulting. This was done on July 6. During the following two days 800 cubic centimeters of urine were passed, containing 4 per cent. of sugar. The glycosuria then disappeared. On July 15 the graft was extirpated. Severe diabetes followed. In 1898 Hédon[7] was able to report only two additional experiments out of a large series in which he tied the pedicle without diabetes resulting.[8]

[3] Hédon, E., *Compt. rend. Soc. de biol.*, 1892, iv, 307, 678; *Arch. de physiol. norm. et path.*, 1892, ser. 5, iv, 617.

[4] Thiroloix, J., *Bull. Soc. anat. de Paris*, 1892, lxvii, 483; *Arch. de physiol. norm. et path.*, 1892, ser. 5, iv, 716.

[5] Pflüger, E. F. W., Das Glykogen und seine Beziehungen zur Zuckerkrankheit, 2d edition, Bonn, 1905.

[6] Pflüger, E. F. W., *loc. cit.*, p. 504.

[7] Hédon, E., Travaux de physiologie, Paris, 1898, cited by Hédon, E., in *Rev. de méd.*, 1910, xxx, 622.

[8] The following abstracts of these two successful experiments are given by Pflüger. We have not had access to the original publication of Hédon.

In a single experiment made in Minkowski's laboratory, Lombroso[9] succeeded in cutting the vascular stalk of a subcutaneous graft without the production of diabetes. This was done one month after the graft was placed under the skin. During this period there were traces of sugar (less than 0.3 per cent.) in the urine. For four days after the operation there was definite glycosuria which at first amounted to 1.5 per cent., but steadily decreased. Twelve days after tying the stalk the graft was excised. Severe diabetes developed and continued until the death of the animal. The success of the experiment was undoubtedly due to a newly formed artery which was found growing into the graft from the skin. This nourished the pancreatic tissue after the original blood supply was shut off.

Martina, an assistant of Payr, made an experiment that has been widely quoted.[10] In discussing a paper by Minkowski, Martina[11] stated that on January 18, 1906, he removed half of the pancreas from a dog and transplanted it in the spleen, using the method that Payr had devised for transplanting the thyroid. The piece of pancreas measured eighteen centimeters in length. The operation was followed by no symptoms. On March 14 the remaining half of the pancreas was extirpated. Sugar appeared in the urine on the second day after the operation. It varied in amount from 3 to 6 per cent.; occasionally it ran as high as 9 per cent. The animal died on July 15 of suppurative peritonitis which took its origin from a fistula in the abdominal wall. At autopsy no pancreatic remains were found at the site of the pancreas. The portion of the organ

Experiment 19.—Subcutaneous transplantation, March 1. After eighteen days total extirpation of the intra-abdominal pancreas and ligature of the mesenteric pedicle of the graft. Transitory glycosuria. After three days urine was free from sugar. Graft extirpated. March 23. Severe diabetes.

Experiment 20.—Subcutaneous transplantation, March 24. After nineteen days (April 12) total extirpation of the intra-abdominal remains of the pancreas and resection of the mesenteric stalk between two ligatures.

From April 13 to 17 only traces of sugar in the urine.

April 17. Extirpation of the graft. Diabetes.

[9] Lombroso, U., abstracted in *Ergebn. d. Physiol.*, 1910, ix, 11.

[10] A detailed report of the work was not published owing to the death of this young investigator. The only description that exists is in the report of a meeting of the Medizinischer Verein in Greifswald.

[11] Martina, *Deutsch. med. Wchnschr.*, 1908, xxxiv, 45.

transplanted in the spleen was "*vollkommen eingeheilt.*" It showed central necroses, but the peripheral cells in contact with the splenic tissue were found on microscopical examination to be intact. As the transplant was entirely separated from the nerve supply of the pancreas Minkowski regarded this experiment as furnishing strong evidence against the neurogenic theory. It should be pointed out that the graft did not prevent the development of glycosuria. In fact the animal had a severe diabetes. It lived the unusually long period of four months after the extirpation of the pancreas, and this would indicate that the pancreatic tissue in the transplant was functionating, provided there was no pancreatic tissue left when the rest of the gland was removed.

The transplantation of pancreatic tissue into the spleen has been attempted by Alessandri,[12] Ottolenghi,[13] Kyrle,[14] and Tiberti.[15] The first three of these investigators described only degenerative changes. Their grafts underwent rapid and complete necrosis. Kyrle also failed in his attempts to keep alive direct implantations of pieces of the pancreas. He had success, however, with a two stage operation. A portion of the duodenal end of the pancreas (probably the processus uncinatus) was freed from its mesenteric attachments and inserted into the spleen. At a second operation the part of the pancreas which had been thrust into the spleen was separated from the remainder of the gland close to the intestine. Dogs as well as guinea pigs were used. The longest interval between the operation and death of the dog was forty days. Kyrle concerned himself with the histological changes in the pancreatic cells and brought forward no evidence that the graft was able to functionate.

We have studied the influence of pancreatic transplants in the spleen on the prevention of diabetes. The spleen was selected because Payr[16] showed that transplanted bits of tissue were especially well nourished in the pulp of this organ. Direct implantations were

[12] Alessandri, R., *Policlinico,* 1896, iii–C., 253; 1897, iv–C., 289; cited by Ottolenghi, D., *loc. cit.*

[13] Ottolenghi, D., *Arch. ital. de biol.,* 1901, xxxvi, 447.

[14] Kyrle, J., *Arch. f. mikr. Anat.,* 1908, lxxii, 141.

[15] Tiberti, N., *Arch. ital. de biol.,* 1909, li, 123.

[16] Payr, E., *Arch. f. klin. Chir.,* 1906, lxxx, 730; *Verhandl. d. deutsch. Gesellsch. f. Chir.,* 1908, xxxvii, pt. 1, 31.

made except in one experiment when a graft was supplied with a vascular stalk. Such an investigation promised to yield information which would either support or oppose the theory of an internal secretion. If diabetes did not occur, it was planned to determine the limit of tolerance for carbohydrates and to keep the animals under observation for a long period. A histological examination of the grafts was undertaken with the hope of obtaining new knowledge on the relation of the islands of Langerhans to the internal function of the pancreas.

Minkowski and Hédon had shown that subcutaneous grafts would prevent for a few weeks the occurrence of diabetes. It was not known that a graft would carry on the internal function of the pancreas for a longer time.

In the experiments with subcutaneous grafts, provision had been made by investigators for the discharge of the external secretion. In three of Minkowski's dogs[17] the pancreatic fistula in the skin failed to develop. In these animals atrophy of the graft was followed by diabetes in thirty-seven days or less. Judging from this experience and that reported by Sandmeyer[18] in his study of the effect of partial extirpation of the pancreas, one would expect that a graft in the spleen, where no escape for external secretion were possible, would be followed by atrophy of the graft and diabetes. Our previous observations[19] had shown, however, that extreme atrophy of the pancreas and complete occlusion of all the pancreatic ducts with retained secretion of the pancreatic juice can occur without diabetes supervening. One dog lived thirty-four

[17] Minkowski, O., *Arch. f. exper. Path. u. Pharmakol., loc. cit.*, pp. 120–134.

Experiment 17.—Graft 8 cm. long placed under the skin, June 24. The graft swelled at first, later became small and hard. Extirpation of intra-abdominal pancreas, July 15. Glycosuria developed, July 22.

Experiment 18.—Subcutaneous graft 5 cm. long, February 17. Extirpation of pancreas, March 7. Diabetes followed. Death, March 14. No trace of graft found.

Experiment 19.—Subcutaneous graft 3.5 cm. long, March 3. After two weeks the graft was so small as to be scarcely palpable. Pancreatectomy, April 6. Diabetes developed, April 9.

[18] Sandmeyer, W., *Ztschr. f. Biol.*, 1895, xxxi, 12.

[19] Pratt, J. H., Lamson, P. D., and Marks, H. K., *Tr. Assn. Am. Phys.*, 1909, xxiv, 266; Pratt, J. H., and Spooner, L. H., *Arch. Int. Med.*, 1911, vii, 665.

months after permanent occlusion of all the pancreatic ducts, and did not develop glycosuria.[20]

In the present study dogs and cats were used. Tissues were fixed in Zenker's fluid or 10 per cent. formalin, imbedded in paraffin, and stained with eosin and methylene-blue, or with hematoxylin and eosin.

DIRECT IMPLANTATION OF PANCREATIC TISSUE IN THE SPLEEN.

Experiment 1.—Female dog; weight, 5,300 gm.

November 8. Operation. Animal had not been fed for twenty-four hours. Ether narcosis. Incision in median line of abdomen. A tight ligature was placed about the processus uncinatus and a transverse section made just distal to the point of constriction. The terminal portion of the processus uncinatus, about 5 cm. long, 2 cm. wide, and 2 to 3 mm. thick, was removed after tying the vessels connected with it. An incision 4 cm. long was made in the lower end of the spleen, and with a sharp knife a pocket was made in the substance of the organ. The peritoneal coat of the excised portion of the pancreas was partly removed. The graft was then buried in the spleen. The wound in the spleen was closed with interrupted sutures. The oozing quickly stopped. The hemorrhage produced a tumefaction about 3 cm. in size beneath the surface of the spleen near the incision.

November 9. Urine voided since the operation, 52 c.c. Specific gravity, 1,058. No sugar. Urine slightly levorotary. The dog is lying quietly in the cage, and took 150 c.c. of warm milk this morning.

November 10. The dog rose to its feet as the cage was approached. 250 c.c. of milk were given. Urine, 250 c.c. Specific gravity, 1,025. No reduction of Fehling's solution.

November 11. The dog is bright and active.

Symptoms of distemper developed on November 15 and the animal died on November 27.

Autopsy.—A small, fairly well nourished dog. Pus exuded from a stitch abscess at the site of the operation wound in the skin. There were small foci of suppuration in the abdominal wall. None of the abscesses were connected with the peritoneal cavity.

The peritoneum was smooth and glistening. The spleen was of normal size and natural appearance externally. Firm adhesions united the omentum to the spleen. The pancreatic transplant was represented by a mass of rather soft, grayish white tissue imbedded in the substance of the spleen. It was separated from the splenic substance by a grayish opaque wall 1 mm. thick. The graft was only slightly adherent to the wall, and on sectioning the organ it fell away leaving a small cavity lined by a white membrane. The splenic tissue about the graft appeared natural. There were no areas of hemorrhage in the spleen.

Microscopical Examination.—Sections through the spleen at the site of the

[20] Pratt, J. H., *Jour. Am. Med. Assn.*, 1912, lix, 322.

graft showed a large area of necrotic tissue surrounded by a zone of pigmented cells. Beyond this was a wall of widely meshed connective tissue. No pancreatic tissue was found.

Experiment 2.—Male dog; weight, 5,000 gm.

November 10. No food for twenty-four hours. Operation under ether narcosis. A portion of the processus uncinatus of the pancreas measuring 5 cm. in length was excised and imbedded in the spleen. After closing the incision in the spleen the omentum was drawn over the wound and sutured to the spleen.

November 12. Rapid recovery from the operation. Urine, 310 c.c. Specific gravity, 1,012. Sugar, 0.55 per cent.; amount, 1.7 gm.

November 14. Urine free from sugar. The dog was sent to the dog farm where it died of distemper on December 6.

Autopsy.—A fairly well nourished dog. Aside from a small subcutaneous abscess the operation wound was completely healed. The peritoneal cavity was dry, the serosa pale and glistening. The omentum was firmly adherent both to the anterior and posterior surface of the spleen. The lower third of the spleen was swollen, measuring 1.5 cm. in thickness, while the remainder was only half this size. The pancreatic graft was represented by a soft, pale yellow body of almost circular outline and measuring from 2 mm. to 1 cm. in width. On the anterior surface the graft was joined to the omental tissue. The spleen was firm and pale red on section.

Microscopical Examination.—The site of the graft was occupied by structureless necrotic material. Between this and the splenic tissue was a zone of granulation tissue containing in its meshes many endothelial cells, some of which contained blood pigment.

Experiment 3.—Male dog; weight, 9,100 gm.

November 29. Operation under ether. The processus uncinatus of the pancreas was tied off between ligatures and the peritoneal covering partially removed. The pancreatic tissue was sliced into strips 6 cm. long, 1 cm. wide, and 0.5 cm. thick. One of these was transplanted into the lower pole of the spleen, and a second piece, with peritoneum attached to one side, was buried in the upper part of the spleen. The incisions in the spleen were closed with interrupted fine linen sutures. The opening in the upper pole of the spleen was longer than usual, making a trough rather than a pocket. The omentum was drawn over incisions and stitched in position. In this operation a dull knife was used. Some tearing of the splenic tissue and excessive hemorrhage occurred. The dog recovered from the operation, but was soon attacked with distemper, and was killed on December 12.

Autopsy.—The body was warm. Rigor mortis was not present. The operation wound had healed. The upper end of the spleen was adherent to the parietal peritoneum, the lower end to the small intestine and its mesentery. The lower part of the spleen was not thickened. The graft was represented by a bit of soft white material about 1 cm. long and 1 to 2 mm. thick. Occupying the site of the upper graft was a mass of fairly firm pinkish tissue 1 cm. in diameter. It was closely adherent to the surrounding splenic substance and reached to the surface of the organ.

Microscopical Examination.—Sections through the spleen showed that the

grafts had been entirely destroyed and converted into necrotic material containing many chromatin fragments and polynuclear leucocytes. Young connective tissue separated the necrotic mass from the splenic tissue.

Experiment 4.—Female dog. November 29. Operation under ether anesthesia. The processus uncinatus was tied off between ligatures. A piece of this, 7 cm. long, 1 cm. wide, and 0.5 cm. thick, was so cut as to be free from peritoneal covering, and was planted in the lower lobe of the spleen. A deep pocket was made and the graft was fixed in place without tension or pressure. The line of incision was covered with omentum.

The dog made a good recovery. As distemper subsequently developed it was chloroformed on December 12.

Autopsy.—The body was warm. The operation wound had healed. The spleen was 10.5 cm. in length. The upper part was thin and narrow, 2 cm. wide, and 6.5 cm. long; the lower part bulbous, 4.5 cm. wide, and 4 cm. long. On section of this swollen part of the spleen a soft red disintegrated substance exuded. There was a zone of grayish red tissue 4 mm. thick surrounding this softened material.

Microscopical Examination.—A bit of pancreatic tissue 1 mm. long and about 0.1 mm. wide was found. It was composed of acini (figure 1). No islands of Langerhans were present. Near this were a few isolated acini imbedded in connective tissue. Most of the acini took the nuclear stain well. There was no leucocytic or lymphocytic infiltration about the pancreatic cells. Separated from this graft by a fibrous wall 1 mm. thick was a large mass of necrotic tissue. Sections from other parts of the pancreatic transplant, showed only necrotic material and young connective tissue.

Thirteen days after transplanting a piece of pancreas into the spleen, normal acini were found. But out of a graft measuring 7 by 1 by 0.5 centimeters only a tiny nodule 1 by 0.1 millimeters remained.

Experiment 5.—Male dog; weight, 9,400 gm.

April 6. First operation, under morphin and ether. The processus uncinatus of the pancreas was sectioned between two ligatures at a point 6 cm. from the distal end. The omentum was placed between the divided portions.

April 13. Second operation. The dog was given morphin subcutaneously before etherization. In the anterior abdominal wall in the vicinity of the first incision was a large amount of chronic inflammatory tissue. The pancreas was of increased consistence and greatly swollen. The terminal portion of the processus uncinatus was closely bound to the remainder of the gland from which it had been separated at the first operation, and the omental tissue which had been placed between the two had almost disappeared. The separation of the two portions was easily made and no connections were found except on the external surface of the gland. The isolated part of the processus uncinatus had undergone considerable atrophy. It was of firm consistence and the surface was granular. It measured 3 cm. in length, 2 cm. in width, and 1.5 cm. in thickness. On section no duct was visible and no hemorrhage or fat necrosis was found. An incision was made in the lower part of the spleen and several thin transverse

sections of the atrophied pancreas were placed in a deep pocket which was prepared in the usual manner. A few grafts were also buried in the upper end of the spleen. The two incisions in the spleen were closed with silk and were left uncovered by omentum. On attempting to separate the corpus pancreatis from the duodenum, the serosa and a portion of the muscular coat were torn away from the wall of the duodenum which was abnormally friable. Owing to the inflamed state of the pancreas the attempt to separate this organ from the gut was abandoned. Several hours after the operation the dog was walking about the cage and in good condition.

April 14. At 9.30 A. M. the dog was found lying on its side and would make no attempt to rise. The animal died half an hour later (eighteen hours after the operation).

Autopsy.—The serosa of the small intestines was dull and lusterless and was covered with elevated lines and nodosities which were apparently produced by irregular contractions of the muscular coat of the intestine. There were about 50 c.c. of bloody fluid free in the peritoneal cavity and considerable hemorrhage about the wound in the abdominal wall and in the mesentery near the pancreas and duodenum. The pancreas was swollen, the surface smooth, and the consistence firmer than normally. The gland surrounded one half of the duodenum. There were no foci of hemorrhage or fat necroses. The duodenum was of normal color and its wall was apparently not inflamed. The spleen was slightly larger than at the time of operation. Scattered over its plum colored surface were a number of darker nodular elevations averaging 1 cm. in size. These marked the direction of the burrows made in the spleen and on section were found to be composed of blood. The two incisions in the spleen were closed by sutures and there was no evidence of any escape of blood from them. The grafts of pancreas placed in the spleen the day before had disappeared. In their place were a few small shreds apparently of clotted blood. The splenic pulp surrounding the pockets appeared normal. The heart, lungs, liver, and kidneys were normal.

Microscopical Examination.—Spleen. The pancreatic transplants had been converted into structureless necrotic material containing fragmented nuclei and hemorrhagic areas; there was no infiltration with polynuclear leucocytes. Here and there between the necrotic material and the splenic tissue were scanty remains of pancreatic tissue. One of these measured 2 mm. in length. The acini in some places stained well and were of normal appearance. In other sections the acini were so distorted that they resembled giant cells.

Experiment 6.—Female dog, strongly built and well nourished; weight, 8,800 gm. April 13. Operation under ether anesthesia. A burrow was made with scissors in both the upper and lower ends of the spleen, and four or five thin transverse sections of the partially atrophied pancreas of the dog used in experiment 5, which had been removed thirty minutes previously and kept in moist gauze, were inserted into the lower pocket in the spleen, and three or four sections into the upper pocket.

The pancreas was separated from the duodenum. Two good sized ducts were found and tied. In making the dissection a large branch of the pancreaticoduodenal artery was cut. The omentum was drawn between the pancreas and the duodenum.

April 14. This morning the dog was walking about the cage and did not seem sick. When seen at 12 o'clock it was apparently in good condition. At 2 P. M. the animal was found dead and rigor mortis was already present (twenty-one hours after the operation).

Autopsy.—There was no evidence of infection about the fresh abdominal wound. The peritoneal cavity was dry and the serosa smooth and glistening. The stomach was moderately distended. There were some petechiæ on its serous coat. The pancreas was soft, but not swollen. There were no hemorrhages into the pancreas, and no fat necrosis. The ducts were not dilated. The spleen was swollen. It measured 13 by 5 by 1.5 cm. Several hemorrhagic areas had produced nodular elevations of the surface. The organ was carefully sectioned, but of the pancreatic grafts placed in the spleen the day before the dog's death, not one could be found. The channels in the spleen made at the operation contained only dark red, soft, homogeneous material, apparently blood and splenic pulp. The heart was distended with blood. The lungs were pale and flaccid. The liver was normal in size and appearance and no focal necroses were seen. The kidneys were normal.

Microscopical Examination.—On section of the spleen not a trace of pancreatic tissue could be found. Degeneration of the acini and a few small areas of fat necrosis were found in the pancreas.

The results in the last two experiments are remarkable. While the grafts of normal pancreas used in the other dogs did not produce any symptoms, the two dogs which had grafts from a pancreas which was the seat of a mild acute pancreatitis died within twenty-four hours. The dog (experiment 5) which had acute pancreatitis, as revealed by the operation, seemed perfectly well prior to the implantation of the pancreatic tissue in the spleen. Yet the transfer of a bit of its own pancreas to the spleen caused speedier death than in the case of the normal dog. This dog (experiment 6) seemed well until less than two hours before its death. Some poisons such as the diphtheria toxin act in this way. A rabbit injected with a lethal dose of diphtheria toxin may not appear sick until less than an hour before death occurs. The implantation of a normal pancreas in the peritoneum causes the death of the animal, but von Bergmann and Guleke[21] found that a preliminary injection of trypsin conferred immunity. On the other hand, our dog, the subject of mild inflammation of the pancreas, appeared very susceptible to the toxic action of the pancreatic grafts, more so in fact than the normal dog. This is a subject that is worthy of further investigation, for it promises to throw new light on the

[21] von Bergmann, G., and Guleke, N., *München. med. Wchnschr.*, 1910, lvii, 1673.

toxic action of the pancreas and the cause of death in acute pancreatitis.

Experiment 7.—Male dog; weight, 8,000 gm.

January 12. Received meat, 300 gm., and glucose, 115 gm.

January 21. Urine, 80 c.c. (acid); specific gravity, 1,036. No sugar.

February 11. Received meat, 300 gm., and glucose, 125 gm.

February 12. Urine, 175 c.c.; specific gravity, 1,050. Sugar, 0.3 per cent.; amount, 0.53 gm.

February 16. Weight, 7,600 gm.

First operation. Ether narcosis. The pancreas was large and of normal appearance. The corpus pancreatis measured 7 by 2.5 cm.; the processus uncinatus, 10 by 2.5 cm.; the processus lienalis, 10 by 2.5 cm. A large pancreatic duct was found on the ventral surface of the corpus pancreatis, midway between the upper and lower boundary of this portion of the pancreas. It was directly behind a branch of the pancreaticoduodenal vein. About 3 cm. above this was a second duct nearly as large. Both were cut between double silk ligatures and the intestinal ends buried in the wall of the duodenum. The pancreas was separated from the duodenum, and the omentum was placed between the two. A portion of the processus uncinatus, measuring 7 cm. in length and 2 to 3 cm. in width, was removed by cutting between double ligatures. This portion was cut into thin longitudinal strips with the blade of a safety razor. Some of these pieces were placed in a pocket made by separating the peritoneum from the upper part of the right rectus muscle near the operation wound. A large deep incision was made in the spleen. A piece of gauze was placed in this wound and time allowed to permit clotting of the blood. A sheet of pancreatic tissue 3 cm. long, 1 cm. wide, and 1 to 2 mm. thick, was pushed into the pocket. There was still some oozing of blood when the grafts were buried in the spleen. The incision in the spleen was closed with sutures and covered with omentum.

Recovery from the operation was rapid.

March 1. Weight, 6,400 gm.

March 6. Received meat, 300 gm., and glucose, 100 gm.

March 7. Urine, 400 c.c.; specific gravity, 1,020. No reduction of Fehling's solution. The dog has a ravenous appetite, but seems weak. The stools have been numerous and of large calibre. They contain a great number of muscle fibers.

March 8. Weight, 5,800 gm. The dog is now unable to jump into the cage. Received meat, 300 gm., and glucose, 125 gm.

March 9, 11 A. M. No urine voided since yesterday.

March 10. 330 c.c. of urine beneath cage; specific gravity, 1,033. No sugar. Received meat, 300 gm., and glucose, 125 gm.

March 11. Urine, 345 c.c.; specific gravity, 1,035; slight trace of albumin. Sugar, 0.2 per cent.; amount, 0.64 gm.

Received meat, 500 gm., and glucose, 100 gm.

March 12. Urine, 265 c.c. No sugar.

March 13. Weight, 6,000 gm.

March 20. Weight, 5,900 gm. Received meat, 500 gm., and glucose, 100 gm. Five hours later 100 c.c. of urine were collected. This contained 4.8 per cent. of sugar.

March 21. Urine voided since yesterday afternoon is free from sugar.

On April 26 the feeding of raw pancreas was begun and continued until May 19.

May 5. Received meat, 300 gm., 3 pancreases, and glucose, 100 gm.

May 6. Urine, 140 c.c. Sugar, 0.6 per cent.

May 23. Weight, 5,900 gm.

August 18. Weight, 5,200 gm. Received meat, 300 gm., and glucose, 100 gm.

August 19. Urine, 370 c.c. Sugar, 3.75 per cent.; amount, 13.87 gm.

August 22. Received meat, 300 gm., and glucose, 50 gm.

August 23. Urine, 180 c.c.; specific gravity, 1,035. A trace of sugar was present. Slight reduction of Fehling's solution on standing.

August 24. Received meat, 300 gm., and glucose, 45 gm.

August 25. Urine, 145 c.c.; specific gravity, 1,035. No sugar.

September 11. Second operation. Morphin-ether anesthesia. The omentum, liver, and upper part of the small intestine, including the duodenum, were found bound together by adhesions. Over the lower third of the spleen the omentum was adherent. After separating the adhesions, the duodenum was brought outside the abdomen, and the region of the pancreas was exposed. The pancreas was represented by a small dense mass attached to the wall of the duodenum 2 to 3 cm. below the pylorus. It was grayish white on section and was apparently composed of scar tissue. Its outlines were indistinct as it merged into the adhesions about the omentum, intestine, and retroperitoneal tissues. Attempts to remove it were abandoned after cutting a large branch of the pancreaticoduodenal artery. The spleen was removed.

September 13. Weight, 4,700 gm. The dog was lively and drank water freely. At 11 A. M. the amount of urine voided since the operation on the day before (eighteen hours) was 85 c.c. It was free from sugar; specific gravity, 1,023.

September 18. Weight, 4,600 gm. The dog was weak. Received meat, 300 gm., and glucose, 40 gm. Vomited a few minutes later.

September 20. Received meat, 300 gm., and glucose, 30 gm. The dog did not eat all the mixture.

September 21. Urine, 70 c.c. It reduced Fehling's solution. 0.2 per cent. sugar was present. The dog ate very little of the 300 gm. of meat and 300 c.c. of milk given today.

September 22. Animal found dead in the cage in the morning.

Autopsy.—Body of a greatly emaciated dog; weight, 4,100 gm. The operation wound was not fully healed. Some thin pus exuded. The anterior abdominal wall was thickened in the region of the incision, measuring 1 to 2 cm. in thickness. It consisted of a grayish white tissue, probably necrotic. It had a worm-eaten appearance due to communicating areas of softening. The inflammation extended through the entire abdominal wall. The edge of the liver, pyloric end of stomach, and the duodenum were firmly adherent to the abdominal wall. The serosa of this portion of the peritoneal cavity was injected and in places was covered with shreds of fibrin. The pancreas was represented by a nodule measuring 2 cm. in length, and 1 cm. from above downwards. On section it was firm and grayish white, and soft in texture, as if composed of connective tissue. Scattered through it were yellowish opaque areas about 1 to 2 mm. in size.

Microscopical Examination.—Serial sections of the spleen through the site of the graft revealed no pancreatic tissue. Many sections of portions of the anterior abdominal wall were examined, but they showed no remains of the pancreatic graft.

On August 25 the dog was able to take forty-five grams of glucose without the production of glycosuria. The spleen was removed on September 11. On September 20 sugar appeared in the urine when less than thirty grams of glucose were taken. This reduction of tolerance may have been produced in various ways. It was due possibly to the removal of functionating pancreatic tissue in the spleen, although no pancreatic cells were recognized on microscopical examination. It is more probable that the lowered limit of assimilation was due to injury of the atrophied pancreas during the operation, and to interference with its blood supply. Another possible cause was the poor general condition of the animal. Hence, no definite conclusion can be drawn from this experiment in regard to the function of the pancreatic grafts. It does, however, confirm in a striking manner the claim of Pratt and Spooner[22] that the occlusion of all the pancreatic ducts produces extreme atrophy of the pancreas and lowered tolerance for glucose.

Experiment 8.—Cat.

March 8, operation under ether narcosis. The pancreas seemed unusually soft. The processus uncinatus, measuring about 5 cm. in length and 1 cm. in width, was cut into pieces, each about 1 cm. long and 3 to 4 mm. thick. The spleen was long and thin. A pocket 4 to 5 cm. deep was made in the lower half of the spleen and in this the pieces of pancreas were placed. There was very little bleeding during this procedure. The incision in the spleen was closed with two silk ligatures. The body of the pancreas was then separated from the duodenum in the usual way.

March 9. The cat lies quietly in the cage and seems sick.

March 10. The cat was found dead this morning.

Autopsy.—The serosa was smooth and glistening. The corpus pancreatis and the processus lienalis were firm and edematous. Areas of fat necrosis 1 to 2 mm. in size and of yellowish white color were present on the mesenteric and omental tissue near the pancreas and on the pancreas itself. The duodenum was firm, pale, and swollen. No hemorrhagic areas were seen, and there was no inflammation about the spleen. The lower half was twice the thickness and twice the width of the upper half of the organ. An elevated hemorrhagic area extended upwards 3 cm. from the incision in the lower part of the spleen. There had been no bleeding into the peritoneal sac. On section of the spleen the pancreatic graft, 5 to 7 mm. wide, was surrounded by splenic tissue with only a thin dark line of hemorrhage between the two.

[22] Pratt, J. H., and Spooner, L. H., *loc. cit.*

Microscopical Examination.—This showed that the pancreatic tissue implanted in the spleen had almost completely disappeared. What had been mistaken for the graft on the gross examination was necrotic material. This was infiltrated with blood. Surrounding a portion of this hemorrhagic area was a thin rim of pancreatic cells. In one section a bit of pancreas 2 by 2 mm. was found. The acini were more or less fused together so that their outline and that of the component cells were indistinct. In places the acinar structure was entirely lost. Nothing was seen that could be identified as an island of Langerhans.

Experiment 9.—Male cat, well nourished.

March 13. Operation under ether narcosis. The large pancreatic duct, entering just below the common bile duct, was dissected out and cut between double ligatures. A second smaller duct was found 1 cm. below the first duct. It was ligated in a similar manner.

The corpus pancreatis was freed from the duodenum, sparing no connecting vessels that passed between the upper half of the corpus pancreatis and the intestine. The processus lienalis was removed. It was 15 cm. long and 1 cm. wide. A long narrow pocket was made in the spleen after making an incision 1 cm. long near its lower pole. The lower half of the spleen was burrowed with scissors upwards for a distance of about 5 cm. and downwards 2 cm. With forceps a strip of pancreas 3 cm. long and 3 cm. wide was taken from the splenic end of the pancreas and inserted into the upper pocket, and a second strip 1.5 cm. long was pushed into the lower pocket. The opening in the spleen was closed with two linen sutures, and no omentum was attached to it.

March 21. The cat was found dead this morning. It had not been well since the operation. Yesterday it refused food and appeared weak and apathetic.

Autopsy.—There was serosanguinolent fluid in the subcutaneous tissue in the vicinity of the incision, but no evidence of pyogenic infection. The peritoneal cavity was dry, and the serosa smooth. The duodenum and pancreas were bound quite firmly to the omentum and mesentery. The wall of the duodenum was intact. The corpus pancreatis was very firm and considerably swollen. It was separated from the duodenum by thickened omentum. This portion of the pancreas was about 1.5 cm. in width, and 3 to 4 mm. thick. The surrounding fat tissue was firmer than normally. In color and consistence it resembled the pancreatic tissue so closely that the outlines of the pancreas could not easily be made out. On the mesentery and omentum near the body of the pancreas were a dozen or more areas of fat necrosis 1 to 3 mm. in size, and about the ligated end of the main pancreatic duct the tissues were softened over an area about 5 mm. in size. The spleen was 9 cm. long. The larger pancreatic graft was represented by a firm yellowish white mass, 5 mm. in diameter and about 2 cm. long, lying in close contact with the splenic tissue which was deep red and firm. The lower pocket was filled with a blood clot.

Microscopical Examination.—The graft in the spleen was necrotic and structureless except for a thin broken line of pancreatic epithelium between the necrotic mass and the splenic tissue. The epithelial cells occurred singly and in small groups which resembled giant cells. No definite acini were seen. Other sections through the site of the grafts showed hemorrhagic and necrotic areas, but no pancreatic cells.

SUMMARY OF THE EXPERIMENTS ON THE IMPLANTATION OF PANCREATIC TISSUE IN THE SPLEEN.

Pancreatic transplants to the spleen underwent rapid autolysis. In five of the nine animals examined eighteen hours to 218 days after the transplantation, no pancreatic tissue was demonstrable. In one dog well preserved acini were found thirteen days after the transplantation. In the other three experiments in which pancreatic cells were present in the spleen, the interval between the operation and the death of the animal varied from eighteen hours to eight days.

Experiment 1.—Dog. Nineteen days after transplantation no pancreatic cells were found.

Experiment 2.—Dog. Twenty-six days after transplantation no pancreatic cells were found.

Experiment 3.—Dog. Thirteen days after transplantation no pancreatic cells were found.

Experiment 4.—Dog. Thirteen days after transplantation a bit of pancreatic tissue measuring 1 by 0.1 millimeters was found. It consisted of acini surrounded by connective tissue. No islands of Langerhans were seen (figure 1).

Experiment 5.—Dog. Eighteen hours after transplantation there was mild inflammation (autoplastic transplant). Only scanty remains of pancreatic tissue were found.

Experiment 6.—Dog. Twenty-one hours after transplantation there was mild inflammation (homoplastic transplant). No pancreatic cells were found.

Experiment 7.—Dog. Two hundred and eighteen days after transplantation there were no pancreatic remains.

Experiment 8.—Cat. Two days after transplantation a thin zone of pancreatic tissue was found. It was composed of acini.

Experiment 9.—Cat. Eight days after transplantation there was a thin layer of pancreatic cells between the spleen and the necrotic material.

PANCREATIC TRANSPLANTS IN THE SPLEEN WITH THE BLOOD SUPPLY TEMPORARILY PRESERVED BY MEANS OF A MESENTERIC STALK.

Experiment 10.—Male dog; weight, 13 kilos.

January 16. Received meat, 300 gm., and glucose, 100 gm.

January 17. Urine, 250 c.c.; specific gravity, 1,026. No sugar.

January 20. Received meat, 300 gm., and glucose, 115 gm.

January 21. Urine, 230 c.c.; specific gravity, 1,030. No sugar.

February 10. Received meat, 300 gm., and glucose, 125 gm.

February 11. Urine, 185 c.c.; specific gravity, 1,052. Sugar, 0.3 per cent.; amount, 0.49 gm.

February 13. First operation. Weight, 11,100 gm. Morphin was given subcutaneously prior to the operation. The processus uncinatus was separated from the remainder of the pancreas at a point 5 cm. from the end by cutting between double ligatures. An incision was made in the mesial surface of the spleen and a large pocket was prepared. Into this was introduced the large pancreatic graft which measured 5 by 3 cm. During the manipulation a considerable amount of blood was lost. Finally all the pancreatic tissue except about 1 cm. of the terminal portion was within the spleen. The mesenteric stalk attached to the end of the processus uncinatus which contained an artery and vein, both of good size, was preserved. The graft was secured in place by sutures and the omentum was drawn around the vascular pedicle.

The dog made a good recovery.

March 7. Second operation. Weight, 9,100 gm. Morphin-ether anesthesia. On opening the abdomen a large mass was found behind the coils of intestine covered with mesentery. This was an abscess occupying the lower part of the spleen. It was opened and there escaped about 30 c.c. of thin pinkish puriform material which had a pleasant odor resembling that of heliotrope. A portion of the pancreatic graft, about 2 cm. in size, projected from the spleen. Its outline could not clearly be made out as it was nearly surrounded by mesentery. The stalk supplying the graft was torn and the vessels were tied. There was no inflammation of the pancreas or duodenum. The stump of the processus uncinatus was healed and free from adhesions to surrounding structures. The duodenum was stripped clean of pancreatic tissue. Two ducts were found and cut between double ligatures. The pancreaticoduodenal artery and vein were spared. The pancreas was removed. The extirpated pancreatic tissue weighed 19 gm.

March 8. The dog is active. Water was given in the afternoon. At 10 A. M. there were 110 c.c. of urine under cage; specific gravity, 1,046. It does not reduce Fehling's solution.

March 9. Urine, 150 c.c. No sugar.

March 10. Urine, 305 c.c. No sugar. Received meat, 300 gm., and milk, 300 c.c.

March 11. No sugar in urine. Received meat, 400 gm., and glucose, 75 gm.

March 12. Urine, 160 c.c. Sugar, 1 per cent.; amount, 2.08 gm.

March 13. Urine, 240 c.c.; specific gravity, 1,055. No sugar. Received meat, 500 gm., and glucose, 50 gm.

March 14. Urine, 230 c.c.; specific gravity, 1,050. Sugar, 0.6 per cent.; amount, 1.38 gm. Received meat, 500 gm., and milk, 300 c.c.

March 15. Weight, 7,900 gm. Urine, 410 c.c.; specific gravity, 1,035. No reduction of copper. Received meat, 500 gm., and glucose, 30 gm.

March 16. Urine, 220 c.c.; specific gravity, 1,050. No sugar. Received meat, 300 gm., and glucose, 60 gm.

March 17. Urine, 190 c.c.; specific gravity, 1,054. Sugar, 0.5 per cent.; amount, 0.95 gm.

March 20. Weight, 8,125 gm.

March 23. Weight, 7,730 gm. An absorption experiment of three days' duration ended today. The dog is weak, but does not seem sick.

March 25. At 11 A. M. the dog received meat, 500 gm., and milk, 300 c.c. At 12.30 P. M. it was found lying in the cage, and was unable to stand when placed on its feet. The animal ate 3 pancreases greedily; none were fed previously to this dog. At 5 P. M. 80 c.c. of urine were collected. It was free from albumin and sugar. When taken out of the cage the dog lay on the floor unable to walk or crawl. It was given 3 more pancreases.

March 26. When seen this morning the dog was standing up in the cage. Received 3 pancreases, meat, 500 gm., and milk, 300 c.c.

March 27. Weight, 7,500 gm. Received meat, 300 gm., 3 pancreases, and glucose, 30 gm.

March 28. Urine, 95 c.c.; specific gravity, 1,056. Sugar, 0.15 per cent.; amount, 0.14 gm. Received meat, 300 gm., 3 pancreases, and glucose, 25 gm.

March 29. Urine, 150 c.c.; specific gravity, 1,050. No sugar.

March 31. Weight, 7,600 gm.

April 4. Weight, 7,150 gm. Received meat, 300 gm., 3 pancreases, and glucose, 100 gm.

April 5. Urine, 225 c.c.; specific gravity, 1,070. Sugar, 6.2 per cent.; amount, 13.95 gm

April 10. Weight, 7,400 gm. The stools are fewer in number and less bulky. The dog has received daily 3 pancreases. He is now stronger and can jump out of his cage and alight on his feet. Received meat, 300 gm., 3 pancreases, and glucose, 40 gm.

April 11. Urine, 65 c.c.; specific gravity, 1,053. No reduction of Fehling's solution.

April 12. Received at 10 A. M. meat, 300 gm., 3 pancreases, and glucose, 50 gm. At 4.45 P. M. 110 c.c. of urine had been voided; specific gravity, 1,046. Sugar, 3.1 per cent.; amount, 3.41 gm.

April 14. Weight, 7,900 gm. Received meat, 300 gm., 3 pancreases, and glucose, 100 gm.

April 15. Urine, 210 c.c.; specific gravity, 1,062. Sugar, 2.8 per cent.; amount, 5.88 gm.

May 1. Weight, 7,600 gm. The dog vomits almost daily. Beginning today he will receive 6 pancreases daily.

May 24. Received meat, 300 gm., bone ash, 50 gm., and glucose, 30 gm.

May 27. Urine, 200 c.c.; specific gravity, 1,039. Sugar, 1.2 per cent.; amount, 2.4 gm. 200 c.c. more of urine were collected at 6 P. M. This was free from sugar.

June 2. Weight, 7,300 gm.

August 17. Weight, 6,900 gm. The dog seems fairly strong. During the past two months and a half he has been given pancreases almost daily. Received meat, 300 gm., 5 pancreases, and glucose, 50 gm.

August 18. Urine, 260 c.c.; specific gravity, 1,034. Sugar, 3.5 per cent.; amount, 9.1 gm.

August 19. Received meat, 300 gm., 5 pancreases, and glucose, 30 gm.

August 20. Urine, 190 c.c.; specific gravity, 1,036. Sugar, 0.85 per cent.; amount, 1.61 gm.

August 20. Received meat, 300 gm., 5 pancreases, and milk, 300 c.c.

August 21. Urine, 400 c.c.; specific gravity, 1,040. Sugar, 0.85 per cent.; amount, 3.4 gm.

August 21. Received meat, 300 gm., and 5 pancreases.

August 22. Urine, 360 c.c.; specific gravity, 1,035. No sugar.

August 22. Received meat, 300 gm., 5 pancreases, and glucose, 15 gm.

August 23. Urine, 300 c.c.; specific gravity, 1,035. No sugar.

August 24. Received meat, 300 gm., 5 pancreases, and glucose, 25 gm.

August 25. Urine, 145 c.c.; specific gravity, 1,050. Sugar, 2.5 per cent.; amount, 3.62 gm.

August 25. Received meat, 300 gm., and 5 pancreases.

August 26. Urine, 360 c.c. No sugar.

September 10. Dog lying in cage. The animal has been ill for five days and has vomited much of his food. Today he was quite powerless, in fact unable to lift his head. He was chloroformed.

Autopsy.—The body was that of a greatly emaciated dog. Weight, 5,710 gm. The omentum was free from fat. The retroperitoneal tissues were stained black (old hemorrhage). In the median line of the peritoneal cavity there was an egg-shaped mass 4.5 by 3.5 cm. This proved to be an abscess cavity which occupied the lower portion of the spleen. A cone-shaped projection of firm grayish white tissue, with its base formed by the wall of the abscess, extended into the spleen for a distance of 2 cm. (figure 2). Its maximum diameter was 5 mm. This nodule received its blood supply from the splenic tissue or from the thick fibrous wall of the abscess. The abscess cavity did not connect with the intestine, although it was adherent anteriorly to the jejunum and posteriorly to the large intestine. No pancreatic remains were found at the site of the pancreas.

Microscopical Examination.—The cone-shaped mass extending from the abscess into the spleen was found to consist of dense fibrous tissue. In the center of this were the remains of the transplant of pancreatic tissue. They consisted of acini scattered over an area about 5 mm. in width and less than 1 cm. in length. Except in a few places where the acini were in close contact, they were separated by connective tissue rich in cells (figure 3). Some of the acini were well preserved and contained a definite lumen. The nuclei were vesicular and situated in the periphery of the cells. The protoplasm was fairly abundant and many of the cells were granular. Some vacuoles were seen. Sections stained with phosphotungstic hematoxylin showed blue staining granules (figure 4) in some of the acinous cells (zymogen granules). Scattered throughout the connective tissue surrounding the pancreatic remains were nerve fibers and occasional ganglion cells. There was no necrotic tissue and no infiltration with polynuclear leucocytes in the vicinity of the pancreatic graft. Between the splenic tissue and the pancreatic remains was a wall of dense fibrous tissue 2 to 3 mm. thick. The largest amount of pancreatic tissue found in any one section measured about 4 by 2 mm. No islands of Langerhans were found in any part of the graft.

This dog lived for 187 days with the internal function of the pancreas maintained by a graft in the spleen entirely separated from its original vascular supply and nervous connections. The interval between the date of the transplantation and the death of the animal was 209 days. Although the tolerance for carbohydrates sank to a low point, persistent glycosuria did not develop. Inanition due to the absence of pancreatic secretion from the intestine was the cause of death. The feeding of fresh pancreas supplied this lack in part and apparently prevented the death of the animal in the third week following pancreatectomy. The limit of tolerance to glucose rose for a short time after the feeding of pancreas was begun, but was at a fairly constant low level from May 24 to August 20. The pancreatic remains at death as determined by serial sections were less than one centimeter in size. The larger part of this small nodule was composed of fibrous tissue. Acini were found, and some of the cells contained well preserved granules staining blue with phosphotungstic hematoxylin and identical in appearance with the zymogen granules of the normal pancreas. As these granules do not occur in the cells of the islands of Langerhans their presence furnishes additional evidence that the pancreatic cells in which they occurred in the graft were acinar cells. No islands of Langerhans were found in any of the sections. If Ribbert's statement is true that only cells that can functionate will live when transplanted, then the presence of normal appearing acinar epithelium in this graft 209 days after the pancreatic tissue was transplanted into the spleen is evidence that the acinar cells can carry on the internal function of the pancreas. This experiment does not disprove the neurogenic theory of diabetes, but it presents additional evidence against it, and in favor of the theory of an internal secretion.

The prevention of diabetes for more than six months by means of the small nodule of sclerosed pancreatic tissue in the spleen makes the accuracy of Martina's observations improbable. If his graft in the spleen remained active, why did sugar appear in the urine two days after the remainder of the pancreas was removed and persist in large amount, 3 to 6 per cent., until the death of the animal? Inasmuch as the animal lived four months with this degree of glyco-

suria it is probable that some pancreatic juice was entering the intestine, secreted from remains of pancreatic tissue so small that they were overlooked at the autopsy. Among the dogs in which we have shut out all the pancreatic secretion from the intestine, marked disturbance of absorption attended with great loss of weight, as shown in this experiment, has always occurred.[23]

Minkowski's dogs with pancreatic subcutaneous grafts did not lose weight after the intra-abdominal pancreatic tissue was removed. This is shown in the following abstracts made from the protocols published in his paper.[24]

The fact that these dogs held their weight after removal of the pancreas seems to be conclusive evidence in the light of our findings that even as skilled an operator as Minkowski left some secreting pancreatic tissue attached to the intestine.

CONCLUSION.

Pancreatic tissue implanted in the spleen and separated from its original vascular and nervous connections can live and functionate for months. A small nodule of pancreatic tissue composed of acini without demonstrable islands of Langerhans prevented the development of diabetes. Death occurred 187 days after the extirpation of the pancreas.

EXPLANATION OF PLATES.

PLATE 51.

FIG. 1. Pancreatic acini found in the spleen thirteen days after direct implantation of a portion of the processus uncinatus of the pancreas. A distinct lumen is present in some of the acini.

[23] Pratt, J. H., Lamson, P. D., and Marks, H. K., *loc. cit.;* Pratt, J. H., and Spooner, L. H., *loc cit.*

[24] Minkowski, O., *Arch. f. exper. Path. u. Pharmakol.*, 1893, xxxi, 123–127.

Experiment 14.—Weight of dog, 15 kilos. Oct. 9, subcutaneous graft. Oct. 29, extirpation of intra-abdominal remains of pancreas. Nov. 24, weight, 15.5 kilos.

Experiment 15.—Weight of dog, 9.6 kilos. Nov. 13, subcutaneous graft. Nov. 23, extirpation of intra-abdominal remains of pancreas. Nov. 30, weight, 10.2 kilos.

Experiment 16.—Weight of dog, 17 kilos. May 4, subcutaneous graft. June 20, extirpation of pancreas. July 19, weight, 17.2 kilos.

FIG. 2. Spleen sectioned transversely and both ends folded back. The direction of the incision is indicated by the dotted line. Fibrous tissue is seen in the center of each cut surface. Near the middle of the fibrous nodule groups of pancreatic acini are found (figure 3). There was an interval of 209 days between the transplantation of pancreatic tissue and the death of the dog. No other pancreatic tissue was found in the body. Diabetes did not develop.

PLATE 52.

FIG. 3. Pancreatic tissue in the spleen 209 days after transplantation. The section is a portion of the fibrous nodule shown in figure 2. There are definite acini but no islands of Langerhans.

FIG. 4. Section of the pancreatic graft shown in figure 2 stained with phosphotungstic hematoxylin. The isolated dots and the masses of fused granules in the protoplasm of the cells are identical in appearance and staining reaction with the zymogen granules of the normal pancreas.

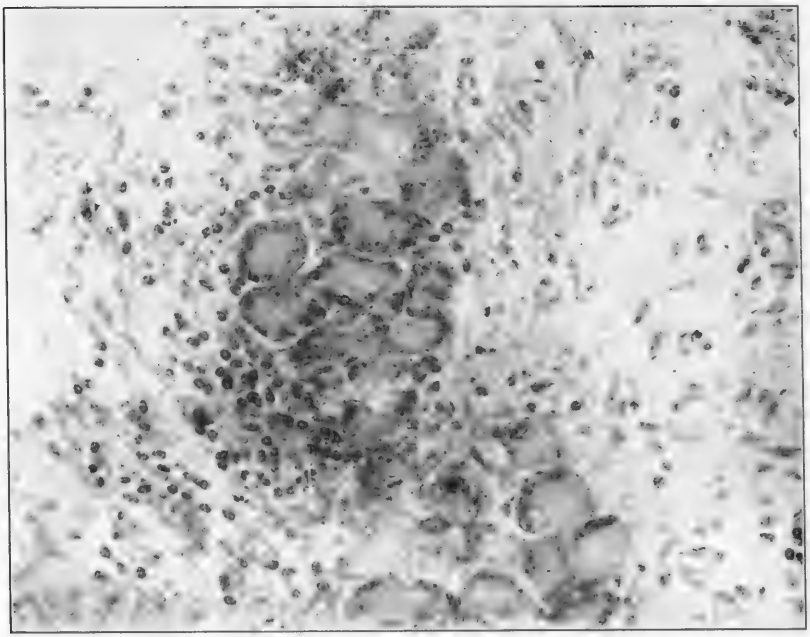

FIG. 1.

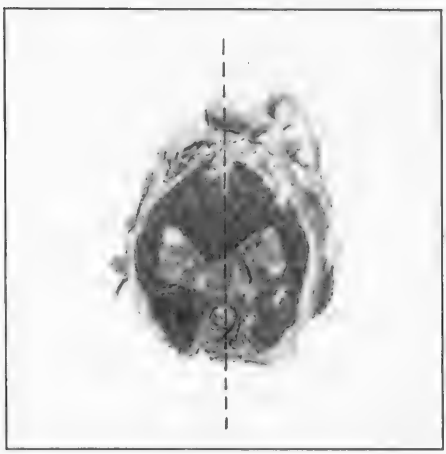

FIG. 2.

(Pratt and Murphy: Pancreatic Transplantations.)

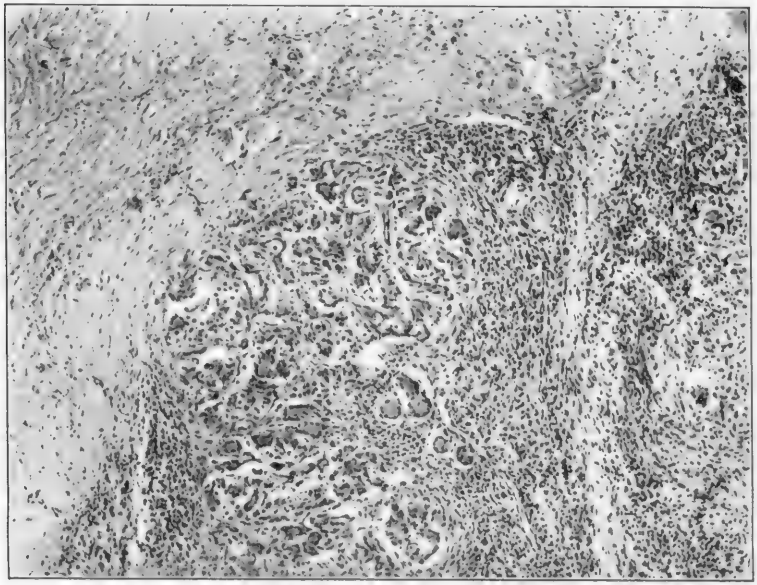

FIG. 3.

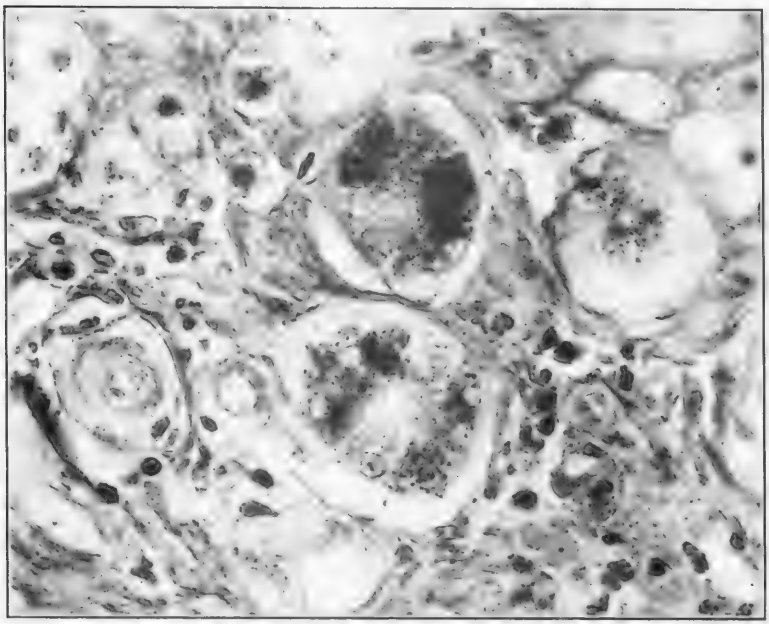

FIG. 4.

(Pratt and Murphy: Pancreatic Transplantations.)

THE PERMANENT LIFE OF CONNECTIVE TISSUE OUTSIDE OF THE ORGANISM.*

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PLATES 53 AND 54.

In previous articles[1] it has been demonstrated that connective tissue cells grow actively outside of the organism for more than four months and that a fragment of heart still pulsates after more than 100 days of life *in vitro*. The purpose of this article is to describe the continuation of these experiments.

The cultures of connective tissue on which Dr. Carrel made his observations were derived from sixteen small fragments of heart and blood vessels, extirpated on January 17, 1912, from chick embryos, seven to eighteen days old. These sixteen cultures did not grow actively. Several accidents occurred, and in March, 1912, only five cultures survived. After some modifications in the technique growth became more active. The mass of the new tissues increased rapidly. The tissues were divided and subdivided. In May, 1912, twenty-five to thirty cultures, derived from the few surviving cultures, were growing actively. The size of the piece of tissue contained in each culture increased very much during the latter part of May, 1912. Large necrotic areas appeared in the center of the tissues, and the growth now became less active.

I took charge of these cultures on June 1, 1912. They were washed in Ringer solution and transferred to a fresh medium every second, third, or fourth day, according to the technique described by Dr. Carrel.[2] The growth was slow and several bacterial infections occurred, so that on July 1 only five cultures survived. These, however, were growing actively. As the fragments were very small it was not possible to divide the tissues. In the beginning of September they were in good condition (figures 1 and 2). Technical accidents caused the loss of several cultures, and on September 25 only one culture survived. This culture arose from a fragment of connective tissue derived indirectly from the fragment of heart

* Received for publication, November 26, 1912.

[1] Carrel, A., *Jour. Exper. Med.*, 1912, xv, 516; Pozzi, F., *Bull. de l'Acad. de méd.*, 1912, lxvii, 475.

[2] Carrel, A., *loc. cit.*

which still pulsated after 104 days of life *in vitro*. It was very small but grew actively. By repeated washings and passages the rate of growth was activated. On October 23 the piece of tissue began to increase rapidly and was divided in two parts. These cultures grew well and quickly and were divided and subdivided again and again. On November 17 the mass of tissue had increased, and twelve large cultures were finally obtained from the culture left in October. In January, 1913, there were thirty cultures.

The tissues contained in these cultures originated, as stated, indirectly from a piece of heart extirpated on January 17, 1912. Their growth was interrupted 128 or 129 times by a short washing in Ringer solution and by passage into a fresh medium. The history of these tissues is briefly described in table I. In December, 1912, the appearance of the cultures was about the same as of those which have been described in another article.[3] From a central mass of coagulated plasma and cells, new tissue, composed of elongated and ramified cells densely matted, grew out concentrically (figures 3 and 4).

The rate of growth following a passage can be seen by comparison with the width of the concentric ring of connective tissue surrounding the original central piece transferred. In many cases the rate of growth was modified by the osmotic tension, the composition of the medium, the manner in which the piece of tissue was cut out for the transfer, and the frequency of the passages. Nevertheless, growth during the eleventh month of the life of these tissues was very much more active than the growth of a fragment of heart of an eight day old chick embryo, and the mass of the tissues increased rapidly.

The experiments show that connective tissue can be kept in a condition of active growth outside of the organism for more than eleven months,[4] that its mass increases considerably, and its power of proliferation, after such a long period, is more active than at the beginning of its life *in vitro*.

[3] Carrel, A., *loc. cit.*

[4] On February 3, 1913, the tissues derived from this culture had undergone their 138th passage and were in a condition of active growth after more than one year of life *in vitro*.

TABLE I.

Passage.	Date (1912).	Treatment of culture.	Observations.
Experiment 725	Jan. 17	Culture of heart tissue from 7 day old chick embryo cultivated in chick plasma.	Jan. 19, pulsations had disappeared.
1	Jan. 19	Washed in Ringer solution for 5 minutes and cultivated in hypotonic chick plasma.[5]	Jan. 19, after transfer the heart fragment pulsated 48 times per minute, and connective tissue cells began to grow. Jan. 25, pulsations had disappeared.
2	Jan. 25	Washed in Ringer solution for 5 minutes and cultivated in hypotonic plasma.	Slow growth.
3	Jan. 30	Washed in Ringer solution for 5 minutes and cultivated in hypotonic plasma.	Slow growth.
4	Feb. 1	Washed in Ringer solution for 5 minutes and cultivated in hypotonic plasma, 2 drops, plus extract, 1 drop.[6]	Feb. 2, active growth.
5	Feb. 5	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma.	Growing.
6	Feb. 7	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma.	Growing.
7	Feb. 9	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma.	Growing.
8	Feb. 12	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma.	Growing.
9	Feb. 13	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma.	Growing.
10	Feb. 16	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma.	Good growth.
11	Feb. 19	Washed in Ringer solution for 4 minutes and cultivated in hypotonic plasma.	Good growth.
12	Feb. 23	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma.	Growing.

[5] Hypotonic plasma consisted of 3 parts of plasma and 2 parts of distilled water.

[6] The extract was prepared from seven day old chick embryo plus 3 drops of Ringer solution.

Passage.	Date (1912).	Treatment of culture.	Observations.
13	Feb. 27	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma.	Good growth.
14	Feb. 28	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma.	Good growth.
15	Feb. 29	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma.	Pulsations reappeared. March 1, 9 A. M., 88 pulsations at 40° C.
16	March 4	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma.	March 5, 9 A. M., 112 pulsations at 39° C.
17	March 8	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma.	Ameboid and elongated tissue cells growing actively. March 8, 2 P. M., 48 pulsations at 40° C. March 13, 60 pulsations at 32° C. before changing culture. Fair growth of new cells.
18	March 13	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma plus a small drop of extract.	Good growth.
19	March 16	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma, 2 drops, plus fresh extract, 1 drop.	12 pulsations at 39° C. before changing culture. (Irregular pulsations, intermittent, series of 4 or 5 pulsations, then long pause.)
20	March 19	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma, 2 drops, plus fresh embryonic extract, 1 drop.	Pulsations slow and irregular.
21	March 22	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma, 2 drops, plus fresh embryonic extract, 1 drop.	124 pulsations at 40° C., again regular and vigorous. 24 to 28 pulsations.
22	March 26	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma, 2 drops, plus fresh embryonic extract, 1 drop.	44 pulsations, vigorous. Large growth of ameboid cells.
23	March 29	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma, 2 drops, plus fresh embryonic extract, 1 drop.	52 pulsations. Large increase in growth of cells.

Passage.	Date (1912).	Treatment of culture.	Observations.
24	April 1	Washed in Ringer solution for 4 minutes and cultivated in hypotonic plasma, 2 drops, plus fresh embryonic extract, 1 drop.	Folding in of piece in culture. April 2, no pulsations. The fragment of heart is imbedded in the middle of a very thick layer of cells and coagulated plasma.
25	April 2	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma, 2 drops, plus extract, 1 drop.	56 pulsations, vigorous after passage. Good growth.
Experiment 1269–2. 26	April 5	Culture 1239 was divided and washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma, 3 drops, plus fresh embryonic extract, 1 drop.	April 7, large growth, 52 pulsations at 39° C.; elongated and ameboid cells. April 8, large growth. The area covered by cells is equal to 70 times the area of fragment of heart tissue.
27	April 8	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma, 2 drops, plus fresh embryonic extract, 1 drop.[7]	April 11, very large growth.
28	April 11	Washed in Ringer solution for 2 minutes and cultivated in hypotonic plasma, 2 drops, plus fresh embryonic extract, 1 drop.	Large growth of fusiform and ameboid cells. April 13, very large growth.
29	April 15	Washed in Ringer solution for 2 minutes and cultivated in same medium.	April 17, large growth of cells.
30	April 18	Washed in Ringer solution for 2 minutes and cultivated in same medium.	April 19, network of elongated cells growing out from central piece of tissue.
31	April 22	Washed in Ringer solution for 4 minutes and cultivated in same medium.	Good growth.
32	April 25	Washed in Ringer solution for 2 minutes and cultivated in same medium.	April 28, good growth.
33	April 27	Washed in Ringer solution for 2 minutes and cultivated in same medium.	April 28, good growth.
34	April 30	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Good growth.

[7] Culture 1269–1 contained a pulsating fragment of heart, culture 1269–2 contained connective tissue cells which had grown out from the heart fragment in forty-eight hours.

Passage.	Date (1912).	Treatment of culture.	Observations.
35	May 3	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Good growth.
36	May 7	Washed in Ringer solution for 3 minutes and cultivated in same medium. (Culture was not extirpated from cover-glass, fresh medium was added.)	May 8, rapid growth.
37	May 10	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Good growth.
38	May 13	Washed in Ringer solution for 3 minutes and cultivated in same medium.	Growing.
39	May 16	Washed in Ringer solution for 3 minutes and cultivated in same medium.	Growing slowly.
40	May 18	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Large growth of ameboid cells.
41	May 20	Washed in Ringer solution for 2 minutes and cultivated in same medium.	May 21, good growth.
42	May 23	Washed in Ringer solution for 3 minutes and cultivated in same medium.	Growing slowly.
43	May 25	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Good growth.
44	May 28	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing actively; elongated and ameboid cells.
45	May 31	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Good growth; elongated and ameboid cells.
46	June 3	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Good growth.
47	June 6	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Good growth; elongated and ameboid cells.

Passage.	Date (1912).	Treatment of culture.	Observations.
48	June 8	Washed in Ringer solution for 2 minutes and cultivated in same medium.	A few cells growing.
49	June 11	Washed in Ringer solution for 2 minutes and cultivated in same medium.	A few cells growing.
50	June 14	Washed in Ringer solution for 2 minutes and cultivated in same medium.	A few cells growing.
51	June 17	Washed in Ringer solution for 2 minutes and cultivated in same medium.	A few cells growing.
52	June 21	Washed in Ringer solution for 2 minutes and cultivated in same medium.	A few cells growing.
53	June 24	Washed in Ringer solution for 2 minutes and cultivated in same medium.	A few cells growing.
54	June 27	Washed in Ringer solution for 3 minutes and cultivated in same medium.	Slight increase in growth.
55	July 1	Washed in Ringer solution for 3 minutes and cultivated in same medium. Ameboid cells growing	Ameboid cells growing.
56	July 3	Washed in Ringer solution for 3 minutes and cultivated in same medium.	July 5, no growth.
57	July 6	Washed in Ringer solution for 3 minutes and cultivated in same medium.	July 8, growing well; ameboid cells predominate.
58	July 10	Washed in Ringer solution for 3 minutes and cultivated in same medium.	No growth.
59	July 12	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma, 2 drops, plus extract, 1 drop.	July 15, growing poorly; more ameboid cells present than elongated cells.
60	July 15	Washed in Ringer solution for 2 minutes and cultivated in normal plasma and extract.	July 17, active growth.
61	July 18	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Active growth of elongated cells; dense portion very small; cells growing out.

Passage.	Date (1912).	Treatment of culture.	Observations.
62	July 22	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Elongated and ameboid cells. Five old cultures still growing.
63	July 25	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Vigorous growth.
64	July 29	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing vigorously.
65	Aug. 1	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Culture 2091 divided into 3 parts; each piece retained a portion of the old mass.
66	Aug. 3	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing poorly.
67	Aug. 6	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing poorly.
68	Aug. 9	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing slowly. Dense central mass; active cells growing out.
69	Aug. 12	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Aug. 12. The older portion of the piece was discarded, the outer zone of active cells being transferred. Aug. 14, very luxuriant growth.
70	Aug. 15	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Aug. 16, growing fairly well.
71	Aug. 19	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Aug. 20, growing slowly.
72	Aug. 21	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing well; elongated cells.
73	Aug. 23	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Luxuriant growth; ameboid cells; network of elongated cells.
74	Aug. 26	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Aug. 26, growing well; infection of various cultures.
75	Aug. 28	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Aug. 31, extensive growth.

Passage.	Date (1912).	Treatment of culture.	Observations.
76	Sept. 3	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Photograph taken Sept. 3, 9.30 A. M., before passage and six days after change into fresh medium. Growing well.
77	Sept. 5	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Sept. 5, cover-glass broken; no infection. Sept. 7, vigorous growth. Sept. 9, growing well.
78	Sept. 9	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing fairly well.
79	Sept. 13	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing vigorously.
80	Sept. 16	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing vigorously.
81	Sept. 19	Washed in Ringer solution for 2 minutes and cultivated in same medium.	Growing vigorously. Sept. 21, growing vigorously.
82	Sept. 23	Washed in Ringer solution for 4 minutes and cultivated in same medium.	Good growth.
83	Sept. 25	Washed in Ringer solution for 3 minutes and cultivated in same medium.	Growing.
84	Sept. 28	Washed in Ringer solution for 3 minutes and cultivated in hypotonic plasma.	Growing.
85	Oct. 1	Washed in Ringer solution for 4 minutes and cultivated in plasma, 2 drops, plus extract, 1 drop.	Good growth.
86	Oct. 4	Washed in Ringer solution for 3 minutes and cultivated in plasma, 2 drops, plus extract, 1 drop.	Good growth.
87	Oct. 7	Washed in Ringer solution for 1 minute and cultivated in plasma, 2 drops, plus extract, 1 drop.	Good growth.
88	Oct. 10	Washed in Ringer solution for 1 minute and cultivated in plasma (1 part plasma, 3 parts water), 2 drops, plus extract, 1 drop.	Good growth.

Passage.	Date (1912).	Treatment of culture.	Observations.
89	Oct. 12	Washed in Ringer solution for 1 minute and cultivated in same medium.	Excellent growth. Oct. 13–15, excellent growth.
90	Oct. 16	Washed in Ringer solution for 1 minute and cultivated in same medium.	Oct. 17, excellent growth of elongated cells.
91	Oct. 19	Washed in Ringer solution for 1 minute and cultivated in same medium.	Oct. 21, very good growth.
92	Oct. 23	Washed in Ringer solution for 1 minute and cultivated in same medium.	Oct. 24, 7 A. M., large growth of scattered cells. Oct. 25, excellent growth. Oct. 25, 9 P. M., large growth.
93	Oct. 26	Washed in Ringer solution for 30 seconds and cultivated in same medium.	Oct. 26, 11 P. M., good growth.
94	Oct. 29	Washed in Ringer solution for 50 seconds and cultivated in same medium.	Excellent growth.
95	Nov. 1	Culture 2611 divided into 2 parts, washed in Ringer solution for 30 seconds, and cultivated in same medium.	Nov. 1, mass had increased to such an extent that culture was divided into 2 parts. Nov. 2, rapid growth. Nov. 4, great increase in mass and density.
96	Nov. 2	Washed in Ringer solution for 35 seconds and cultivated in same medium.	Nov. 2, 6 P. M., excellent growth 9 hours after changing into new medium. Nov. 4, excellent growth, very dense.
97	Nov. 5	Culture 2647 divided into 2 parts, washed in Ringer solution for 35 seconds, and cultivated in same medium.	Nov. 5, cultures were divided again, making 4. Excellent growth. Nov. 6, infection.
98	Nov. 7	Culture 2662 divided into 2 parts; infected area removed. Washed in Ringer solution for 35 seconds and cultivated in same medium.	Nov. 7, cultures divided again, making 8. Excellent growth.
99	Nov. 9	Culture put directly into new medium without washing.	Growing fairly well.
100	Nov. 12	Washed in Ringer solution for 25 seconds and cultivated in same medium.	Excellent growth.

Passage.	Date (1912).	Treatment of culture.	Observations.
101	Nov. 14	Culture 2734 divided into 2 parts, washed in Ringer solution for 30 seconds, and cultivated in same medium.	Nov. 14, cultures divided again, but cultivated on the same cover-glass.
102	Nov. 17	Culture 2765 divided into 2 parts, washed in Ringer solution for 40 seconds, and cultivated in same medium.	Nov. 17, cultures divided again, making 16 separate cultures. Excellent growth.
103	Nov. 19	Washed in Ringer solution for 1½ minutes and cultivated in same medium.	Excellent growth.
104	Nov. 21	Washed in Ringer solution for 30 seconds and cultivated in same medium.	Nov. 22, excellent growth.
105	Nov. 23	Washed in Ringer solution for 35 seconds and cultivated in same medium.	Nov. 24 and 25, excellent growth.
106	Nov. 25	Washed in Ringer solution for 38 seconds and cultivated in same medium.	Nov. 25, 2 P. M., 3 hours after change, many elongated cells growing out. Nov. 26, excellent growth. Photograph taken.
107	Nov. 27	Washed in Ringer solution for 30 seconds and cultivated in same medium.	Nov. 28 and 29, excellent growth, dense network of cells.
108	Nov. 30	Washed in Ringer solution for 30 seconds and cultivated in same medium.	Dec. 1 and 3, excellent growth.
109	Dec. 3	Washed in Ringer solution for 40 seconds and cultivated in same medium.	Dec. 4, excellent growth.
110	Dec. 4	Washed in Ringer solution for 30 seconds and cultivated in same medium.	Dec. 5, excellent growth.
111	Dec. 5	Culture partially divided, washed in Ringer solution for 30 seconds, and cultivated in same medium.	Dec. 5, 3.50 P. M., excellent growth 5 hours after transfer.
112	Dec. 7	Culture partially divided, washed in Ringer solution for 35 seconds, and cultivated in same medium.	Dec. 8, excellent growth, quite dense.
113	Dec. 10	Washed in Ringer solution for 1 minute and cultivated in same medium.	Dec. 11, excellent growth.

Passage.	Date (1912).	Treatment of culture.	Observations.
114	Dec. 12	Washed in Ringer solution for 30 seconds and cultivated in normal plasma, 2 drops, plus extract (24 hours old and kept in cold storage), 1 drop.	Dec. 13, excellent growth.
115	Dec. 14	Washed in Ringer solution for 35 seconds and cultivated in normal plasma, 2 drops, plus extract (24 hours old), 1 drop.	Dec. 14, 1 P. M., new cells growing out 3 hours after transfer.
116	Dec. 16	Culture divided into 2 parts, washed in Ringer solution for 30 seconds, and cultivated in same medium.	Dec. 19, excellent growth.
117	Dec. 17	Culture divided into 2 parts. Nos. 1 and 2 washed in Ringer solution for 30 seconds. No. 1 cultivated in normal plasma and old extract (prepared Dec. 15).	Dec. 17, 6 P. M., excellent growth; a crown of new cells growing rapidly. Dec. 18, A. M., excellent growth, quite dense.
118	Dec. 19	Washed in Ringer solution for 30 seconds and cultivated in same medium.	Dec. 19, 6 P. M., excellent growth. Dec. 20, A. M., excellent growth, fairly dense.
119	Dec. 21	Culture divided, washed in Ringer solution for 40 seconds, and cultivated in same medium.	Dec. 22, excellent growth, quite extensive.
120	Dec. 24	Culture divided, washed in Ringer solution for 35 seconds, and cultivated in plasma, 2 drops, plus fresh extract, 1 drop.	Dec. 26, excellent growth; area increased about four times.
121	Dec. 27	Culture divided, washed in Ringer solution for 30 seconds, and cultivated in same medium.	Dec. 28, poor growth. Dec. 29, growing slowly. Dec. 30, good growth; many scattered cells grow in a medium due to extract.
122	Dec. 30	Culture divided, washed in isotonic[8] solution for 30 seconds and cultivated in plasma, 2 drops, plus old extract (prepared Dec. 21 and kept in cold storage), 1 drop.	Jan. 2, excellent growth; increase in mass about 4 times original size.

[8] The isotonic solution consisted of sodium chloride, 8.5 gm.; sodium bicarbonate, 0.3 gm.; calcium chloride, 0.25 gm.; potassium chloride, 0.1 gm.; and distilled water to make 1,000 gm.

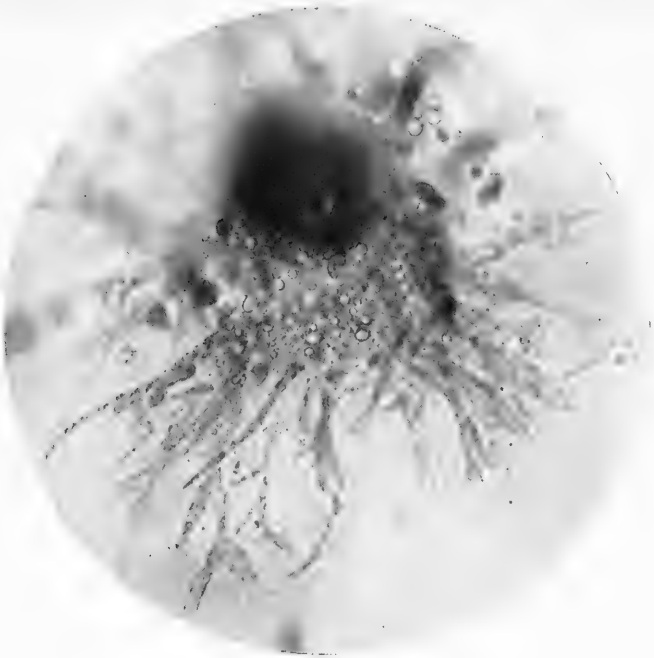

FIG. 1.

FIG. 2.

(Ebeling: Life of Connective Tissue.)

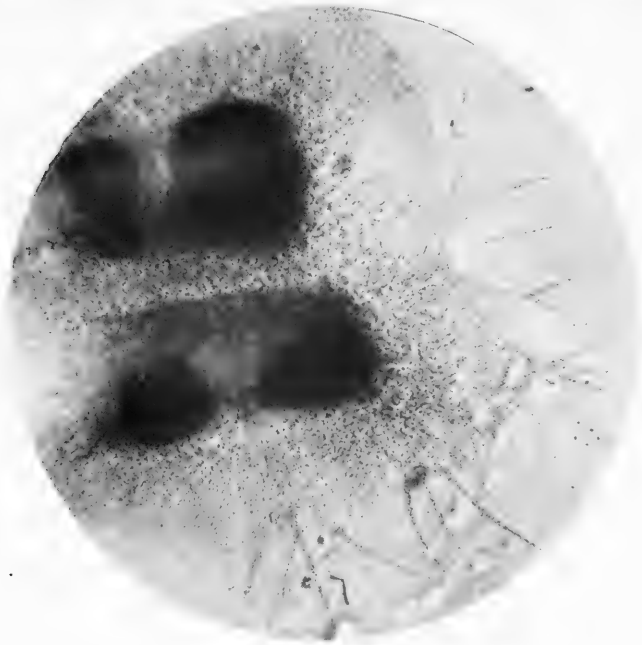

FIG. 3.

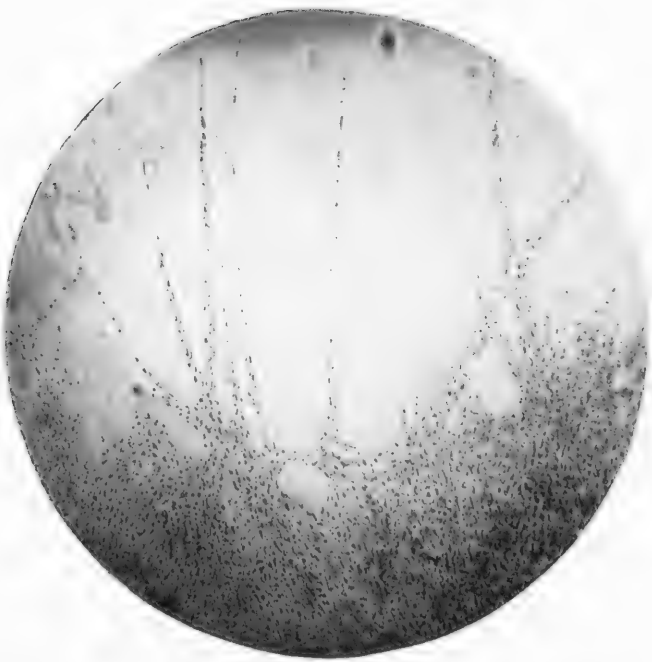

FIG. 4.

(Ebeling : Life of Connective Tissue.)

Passage.	Date (1913).	Treatment of culture.	Observations.
123	Jan. 2	Culture divided into 2 parts, washed in isotonic solution for 35 seconds, and cultivated in plasma, 2 drops, plus old extract (prepared Dec. 27), 1 drop.	Jan. 2, before passage. Excellent growth; increase in mass about 4 times.
124	Jan. 4	Washed in isotonic solution for 30 seconds and cultivated in plasma, 2 drops, plus old extract (prepared Jan. 3), 1 drop.	Jan. 5, good growth.
125	Jan. 6	Washed in isotonic solution for 30 seconds and cultivated in plasma, 2 drops, plus brain extract (prepared from brain of adult chicken, Dec. 19), 1 drop.	Jan. 7, good growth.
126	Jan. 9	Washed in Ringer solution for 30 seconds and cultivated in plasma, 2 drops, plus fresh extract, 1 drop.	Jan. 10, growing fairly well.
127	Jan. 11	Washed in Ringer solution for 35 seconds and cultivated in same medium.	Jan. 12, growing.
128	Jan. 14	Washed in isotonic solution for 1 minute and cultivated in same medium.	Jan. 15, growing fairly well.
129	Jan. 15	Washed in Ringer solution for 30 seconds and cultivated in same medium.	Jan. 15, 4.30 P. M., new cells growing forth. Jan. 16, 9 A. M., very good growth.

EXPLANATION OF PLATES.

Plate 53.

Fig. 1. A culture of connective tissue derived from embryonic heart tissue (chick) which was extirpated on January 17, 1912. The photograph was taken on September 3, 1912, six days after the seventy-fifth passage.

Fig. 2. The same as figure 1; high magnification.

Plate 54.

Fig. 3. A culture of connective tissue derived from the same culture as figure 1. The photograph was taken on November 25, 1912, forty-eight hours after the 105th passage. The photograph shows an opaque area composed of cells and old plasma which is surrounded by a clearer area of the tissue formed in forty-eight hours.

Fig. 4. The same as figure 3; higher magnification. The photograph shows part of the area of tissue formed in forty-eight hours.

INTESTINAL OBSTRUCTION.

I. A Study of a Toxic Substance Produced in Closed Duodenal Loops.*

By G. H. WHIPPLE, M.D., H. B. STONE, M.D., and B. M. BERNHEIM, M.D.

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Plate 55.

During the past few years a great amount of experimental work has been done in an attempt to solve some of the problems concerned with intestinal obstruction. We shall make no effort toward a critical review of this material, but refer to recent publications by Hartwell and Hoguet, Murphy and Vincent, Clairmont and Ranzi, Albeck, Kukula, and Draper-Maury.

Before we proceed with our own work, it will be well to emphasize two points which seem to be fully established by several workers. Clairmont and Ranzi, followed by Hartwell and Hoguet, have shown that blood cultures, cultures from the viscera, and, most important of all, peritoneal cultures, may be sterile when the experimental obstruction has been carefully performed, yet the animals die in the characteristic manner. This rules out the importance of bacterial growth and activity in the general circulation or in the peritoneal cavity, but does not exclude bacterial activity in the intestinal lumen.

Experiments of Halsted and von Baracz show that the closed loops of ileum and colon may be very slightly toxic, the dogs living for weeks or even months. At autopsy the loops were filled with inspissated, cheesy material. Our few experiments with closed loops of ileum give no evidence of grave intoxication and support the observations just cited.

We felt that experiments (Kukula, Murphy and Vincent, and others) in which intestinal contents were introduced into a normal

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animal (intravenously, intraperitoneally, or subcutaneously) were open to objection because of the complexity of the factors involved; namely, bile, pancreatic and gastric juices, besides the split products of proteid digestion which are known to be more or less toxic when given intravenously.

The use of a closed washed duodenal loop does away with some of these confusing factors and when produced in a dog will usually lead to death in one to three days, the symptoms being similar to those of patients with volvulus or high intestinal obstruction; these are intoxication, low temperature, a fall in blood pressure, and venous congestion. By the use of the closed washed loop we are able to exclude bile, gastric and pancreatic juices, and products of food digestion. This procedure simplifies the problem and limits us to a consideration of substances elaborated by the bacteria which are known to multiply rapidly in these closed loops (McClure) and by the intestinal mucosa which is capable of secretion as well as of absorption.

This closed duodenal loop we may compare with a volvulus in which there are no vascular disturbances and no food products to complicate the picture. We hope to show clearly that in such a duodenal loop there is formed a toxic substance, or substances, which give a characteristic picture when introduced into a normal animal of like species. Further, we believe that the intestinal epithelium is necessary for the production of this toxic substance and that dogs can be immunized against this poison. Immunized in this way they may live six or seven days with a closed duodenal loop, which is three times the average length of life under these conditions. Many experiments on dogs with closed duodenal loops are given because it is important to know the history of the cases and the findings under different conditions and after different intervals of time. The material used for study was obtained in large part from these dogs and references to the protocols will be made constantly in the later experiments.

METHOD.

The operative detail with illustrations has been given in a previous communication by the writers. The essential feature, however, is the isolation of the greater part of the duodenum by means of heavy ligatures placed just below

the pancreatic duct and just beyond the duodenojejunal junction. These heavy ligatures are buried by mattress sutures and the continuity of the alimentary tract is established by a posterior gastro-enterostomy. Ether was invariably used as an anesthetic in all operations, and morphia was usually not given. All the experiments were performed upon dogs. The material obtained from the closed duodenal loops at autopsy was usually diluted with normal saline or washed into a container with water. Autolysis with toluol and chloroform does not injure the toxic substance and is valuable for making the material more suitable for filtration or extraction. The fluid, unless otherwise stated, was heated at 60° to 70° C. for one half to one hour, centrifuged, and the supernatant fluid filtered through a Gooch crucible or porcelain perforated funnel. The kymograph experiments were always done under ether anesthesia. The carotid artery and jugular vein can be reached by a small incision, and if the operation is done with clean instruments, and with as little handling of the tissues as possible, the wound will heal promptly.

EXPERIMENTAL OBSERVATIONS.

In the first series of four dogs the isolated loop was not washed out and the intestine was cut across with inversion of the ends. These dogs all died in twenty-four to twenty-eight hours, as described in our previous report, and it is possible that the cut and inverted ends of the intestine favored absorption and the fatal outcome. It may be thought, too, that careful washing out of the duodenal loop before closure in later experiments removed all food elements and great numbers of bacteria, thus retarding the ultimate growth of the bacteria which may be in part essential to the production of the toxic substances. For this reason it will be seen below that in this second series the dogs lived one to three days after operation, or an average of about forty-eight hours

CLOSED DUODENAL LOOP. DEATH IN TWENTY-SEVEN HOURS.

Dog S–34.—Adult mongrel, male; weight, 15 pounds.

April 2, 11 A. M. Operation and isolation of duodenal loop with careful washing.

April 3, A. M. Dog seems very toxic; refuses food. 2 P. M. Dog is moribund. Muscular tremors are very conspicuous. The pupils are dilated. The extremities are cold. Given ether.

Autopsy.—Performed at once. The heart continued to beat with a very slow rhythm several minutes after opening the thorax. The lungs are rather voluminous and pale. The spleen, liver, and kidneys are normal. The duodenal loop is of enormous size. Its serous surface shows a fine granular deposit of fibrin, but elsewhere the peritoneal surface is smooth. Purplish patches show through the transparent serosa. It contains 130 c.c. of blood-stained fluid of a

characteristic odor. Flakes of mucus and epithelium can be made out in the fluid, which is rather thin. The stomach is decidedly pale. The duodenum above the loop shows a pinkish mucosa. The jejunum shows a mottled pinkish red mucosa and contains a good deal of fluid. The splanchnic vessels are conspicuous in the mesentery.

Microscopical Examination.—The lungs, liver, small intestine, and kidneys are normal except for slight congestion. In places the wall of the duodenum shows necrosis, hemorrhage, and invasion by leucocytes. Bacteria are very numerous throughout the necrotic mucosa. The peritoneum shows a beginning exudate of leucocytes.

CLOSED DUODENAL LOOP WITH RUPTURE. DEATH IN THIRTY-TWO HOURS.

Dog S-27.—Large mongrel, male; weight, about 45 pounds.

March 18, 3 P. M. Operation and isolation of closed duodenal loop with washing as usual.

March 19, 11 P. M. Dog found gasping for breath, with marked muscular twitchings. Death followed in a few minutes.

Autopsy.—Performed at once. The peritoneal cavity contains turbid, gray fluid similar to that often found in the closed loops. The peritoneal surfaces are injected and specked with ecchymoses. The duodenal loop is contracted and contains a little material. Its wall shows a few areas of subserous hemorrhage about 1 or 2 cm. in diameter. There is obvious necrosis associated with these areas and through one of them rupture had taken place, with escape of duodenal loop fluid into the peritoneum. The mucosa of the loop in general is intact, but shows some superficial erosion, particularly in association with submucous hemorrhage.

CLOSED DUODENAL LOOP. DEATH IN FORTY-EIGHT HOURS.

Dog S-20.— Large male; weight, about 50 pounds.

February 25, 11 A. M. Operation and isolation of duodenal loop with washing in the usual way. 4 P. M. Dog walks about normally.

February 26. Dog appears sick and has diarrhea with blood in stools.

February 27, 10 A. M. Same, and much vomiting. 11.30 A. M. Death preceded by slow deep gasping respirations; wide pupils and muscular tremors.

Autopsy.—Performed at once. The heart, lungs, and thorax are negative. The peritoneal cavity shows a very slight amount of fibrinous exudate and a few ecchymoses about the area of operation. There is no fluid in the peritoneum. The liver is congested and deep purple in color. The spleen, pancreas, and kidneys show congestion. The stomach contains some peculiar black fluid much like that in the closed loop, and it is probable that the upper ligature was not tight and some of the duodenal fluid has escaped into the stomach. The small intestine shows a diffuse reddening of the mucosa, which is specked with ecchymoses.

The duodenal loop contains 100 c.c. of dark, slaty, slimy fluid. The closed loop mucosa is pinkish and a little swollen, but no ulcers are present.

CLOSED DUODENAL AND JEJUNAL LOOPS. DEATH IN FIFTY-FIVE HOURS.

Dog 56.—Strong, adult male hound; weight, about 45 pounds.

May 22, 3 P. M. Operation and isolation of a loop much longer than usual,

including the duodenum below the pancreatic duct and about six inches of the jejunum. The loop was washed out thoroughly before closure.

May 23. Dog seems well, but has had some diarrhea.

May 24, A. M. Dog vomits bile-stained fluid repeatedly; this vomiting is much more conspicuous than in the usual closed duodenal loop. Dog drinks much water. Rectal temperature, 38° C. 4 P. M. Vomiting continues; marked bloody diarrhea. 11 P. M. Found dead but quite warm.

Autopsy.—Performed at once. The abdominal cavity is clear, except for slight granular peritonitis about the loop and area of operation. The gastro-enterostomy is patent. The stomach contains bile-stained, watery fluid. The small intestine below the loop contains blood-stained mucus, and its mucosa has a mottled appearance with large areas of deep red color, where the capillary injection is quite marked.

The duodenal loop is rather flabby and not greatly distended. It contains about 50 c.c. of a thin, soup-like, faintly blood-tinged fluid. The loop mucosa is quite intact, but of a delicate pinkish tinge.

CLOSED DUODENAL LOOP. DEATH IN THREE DAYS.

Dog S–53.—Mongrel, male; weight, 17 pounds.

May 14, 3 P. M. Operation and isolation of duodenal loop after careful washing.

May 15, A. M. Dog seems normal. 5.30 P.M. Dog lies on side and is quite sick. Temperature, 38° C. Pulse is normal. 8.30 P. M. Dog vomited turbid brown fluid. Pulse is weak. Temperature, 37.5° C.

May 16, 10.30 A. M. Dog is quiet. Temperature, 37° C. 12.30 P. M. Temperature, 36.5° C. Muscular tremors; respirations becoming slow and deep. 5 P. M. Temperature, 38.2° C. Pulse fair. Drinks water and does not vomit. Muscular tremors still present.

May 17, 9 A. M. Vomited during night, and vomits immediately after drinking water. Pulse weak and slow. Temperature, 35.5° C. 10 A. M. Moribund; etherized.

Autopsy.—Performed at once. Blood showed delayed coagulation. The thorax is normal. The peritoneal cavity is normal. The liver, spleen, and mesenteric vessels are congested. The stomach contains a little bile-stained fluid; pyloric portion pale, and cardiac mucosa deep pinkish.

The duodenal loop contains a dark slaty paste, about the consistence of butter, which scrapes off very easily from the pink duodenal mucosa which is intact.

The duodenum just below the pylorus shows definite congestion of the mucosa giving a red mottled appearance. There are no ecchymoses. The injection begins 1 cm. below the pyloric ring and suggests the picture following injection of duodenal fluid in the normal dog. The jejunum below the gastro-enterostomy shows considerable injection of its mucosa, presenting a reddish mottled appearance fading to normal mucosa in the ileum. The intestinal contents are thin, watery, and bile-stained.

This group shows the characteristic picture following the production of a closed washed duodenal loop in a normal dog. The

last case illustrates the drop in temperature. Loss of fluid by diarrhea or vomiting may be conspicuous and is usually obvious. Muscular tremors, twitching, and irritability are usually present. A weak pulse with low blood pressure is present in the last stages. The autopsy shows splanchnic congestion and, usually, dilatation of the closed loop, but this last may not always occur. The intestinal mucosa usually shows the splanchnic congestion to best advantage and is similar to, though much less striking than the extreme engorgement of the intestinal mucosa which may follow the intravenous injection of large amounts of duodenal loop fluid. The duodenal loop may show a normal intact mucosa in gross and microscopically, except for a little diffuse injection. Again it may show necrosis, ulceration, and hemorrhage, and the contained fluid may vary correspondingly in characteristics, but it always contains a toxic substance.

ECK FISTULA AND CLOSED DUODENAL LOOP. DEATH IN THREE DAYS.

Dog C–39.—Large mongrel, male; weight, 37 pounds.

March 2. Operation with production of Eck fistula below the splenic vein, allowing the greater part of the intestinal venous blood to pour directly into the vena cava. A ligature was placed on the portal vein just below the splenic vein.

March 30. Animal in good condition. Operation and ligation of hepatic artery in the usual way.

April 23, 2 P. M. Dog in excellent condition. A closed duodenal loop was made in the usual way, with careful washing out of the intestinal contents. There was some difficulty on account of old adhesions.

April 24. Dog appears active and practically normal.

April 25. Dog is quite sick and refuses food; vomits bile-stained material.

April 26, 10 A. M. Dog is very sick. Pulse weak. Rectal temperature, 38.8° C. Etherized; bled from the carotid which showed very low arterial pressure.

Autopsy.—Performed at once. The abdominal wound shows some infection, but the peritoneal cavity is clear. The lungs show a few tiny patches of bronchopneumonia. The heart, spleen, pancreas, and kidneys are normal. Eck fistula is widely open, and the hepatic artery is completely cut off by the ligatures. The liver is slightly decreased in size as is usual, but is normal except for some capsular adhesions. The stomach contains a little bile-stained fluid and mucus. The small intestine is negative.

The duodenal loop is moderately distended with fluid. The serous surface is quite normal. The fluid is turbid gray in color and of the consistence of pea soup. The duodenal loop mucosa is a diffuse pink, and there is obvious engorgement of the vessels in the mucosa. There are no ulcers nor subcutaneous ecchymoses. The mucosa is intact throughout.

Microscopical Examination.—The mucosa of the duodenal loop is normal

except for a trifling congestion of the capillaries in the villi. The epithelium is normal and intact. The mucus covering the surface is extremely rich in bacteria, and this material contains a good deal of desquamated epithelium. The liver cells appear normal. There is no increase in connective tissue. The presence of the Eck fistula seemed to affect in no important way the general progress of the intoxication.

If the liver exerts a detoxifying action against this poison one might expect a more rapid intoxication in a dog with a closed loop when the intestinal blood is shunted directly into the vena cava (Eck fistula), or less intoxication when the toxic substance is introduced into the portal vein of a normal dog. The preceding experiment speaks against any such protective action of the liver, for the dog with a closed loop and with an Eck fistula survived longer than the average dog with a closed loop alone. Experiments given below show that the duodenal loop material is just as toxic whether injected into the portal vein or into the systemic veins.

CLOSED DUODENAL LOOP AND INTESTINAL OBSTRUCTION. KYMOGRAPH.

Dog C–19.—Mongrel pup, female; weight, 17 pounds.

March 27. Usual operation and isolation of washed duodenal loop.

March 29, 9 A. M. Dog is cold and toxic; would probably die in a few hours. Etherized, and kymographic tracings taken from the carotid. Blood pressure is low, averaging between 70 and 80 mm. of mercury. The pulse rate is very slow, averaging between 60 and 80 per minute, becoming slowed to even 30 or 40 per minute with occasional missed beats. Respiration is slow, about 5 or 6 to the minute, and the tracings showed conspicuous muscular tremors. The dog has cold, livid extremities, and seems in the last stages of surgical shock.

Autopsy.—Performed at once. The peritoneal cavity is clear. The stomach contains about two quarts of bile-stained fluid, caused by a twist in the jejunum just below the gastro-enterostomy, producing considerable intestinal obstruction. The duodenal loop is flaccid and its walls appear normal. It contains a little blood-stained pasty mucoid material in which are found a few small particles of food, showing that the washing had not been sufficiently prolonged. The mucosa is intact and, except for a slight injection, normal. The heart continued to beat for several minutes after the thorax was opened. It is normal. The lungs show a small patch of bronchopneumonia. The other viscera are normal. Since dogs with an intestinal obstruction in this location will live from seven to ten days, it is fair to suppose that the obstruction and especially the duodenal loop were responsible for death.

CLOSED DUODENAL LOOP. KILLED AFTER TWENTY-FOUR HOURS.

Dog S–4.—Adult mongrel, male; weight, 20 pounds.

January 19, 3 P. M. Operation and isolation of duodenal loop with careful

washing. January 20. Somewhat dull but not very toxic. 3 P. M. Ether anesthesia and bleeding from carotid.

Autopsy.—Performed at once. The serous surfaces and viscera are normal. The stomach contains bile-stained fluid and food. The intestine is normal. The duodenal loop is slightly dilated and contains 20 c.c. of slimy fluid which is slightly blood-tinged, due to inversion of the duodenal mucosa. The mucosa throughout the loop is intact, but slightly swollen and of a pinkish color. There are no ecchymoses to be seen.

Microscopical Examination.—The liver and kidneys are normal. The mucosa of the duodenal loop is practically normal. The tips of a few of the villi are slightly swollen and contain a few polymorphonuclear leucocytes. The epithelium is normal throughout.

CLOSED DUODENAL LOOP. KILLED AFTER TWENTY-FOUR HOURS.

Dog *S–28.* March 20, 3 P. M. Operation and isolation of duodenal loop as usual with washing.

March 21, 3.30 P. M. Dog is dull and sick. Ether anesthesia and bleeding from carotid.

Autopsy.—Performed at once. The stomach contains fluid and food. The intestine contains a moderate amount of fluid and shows a rather pale mucosa. The other viscera are negative. The closed duodenal loop contains only 2 to 3 c.c. of pasty, tenacious material. The mucosa shows a definite pinkish tinge, due to injection of the vessels, and is intact everywhere.

CLOSED DUODENAL LOOP. KILLED AFTER TWENTY-SIX HOURS.

Dog S–2.—Mongrel terrier, male; weight, 10 pounds.

January 10, 1 P. M. Operation and isolation of duodenal loop without washing.

January 11, 10 A. M. Dull and drowsy; no vomiting. 3 P. M. Weak. Ether anesthesia and bleeding from carotid which shows low blood pressure.

Autopsy.—Performed at once. The serous cavities and viscera are normal. The stomach contains bile-stained, blood-tinged fluid. The small intestine is normal. The duodenal loop contains 35 c.c. of blood-tinged thick fluid. The mucosa is intact, but seems somewhat swollen. There are a few subserous hemorrhages.

Microscopical Examination.—The liver and kidneys are normal. The duodenal loop in places shows some areas of necrosis with hemorrhage and invasion by pus cells, involving mucosa and submucosa.

CLOSED DUODENAL LOOP. KILLED AFTER TWENTY-EIGHT HOURS.

Dog S–29.—Large mongrel bull, male; weight, 25 pounds.

March 22, 12 M. Operation and washed duodenal loop isolated.

March 23, 4 P. M. Dog appears sick; muscular tremors present. Pulse is strong. Ether and bleeding from carotid.

Autopsy.—Performed at once. The organs are all negative except the duodenal loop which is flabby and collapsed. The serous surface is normal. It contains about 5 to 10 c.c. of thick, viscid, dark brown, mucoid material. The duodenal mucosa appears normal, and shows no injection, ecchymoses, or ulceration.

CLOSED DUODENAL LOOP. KILLED AFTER TWENTY-EIGHT HOURS.

Dog S–5.—Mongrel, male; weight, 14 pounds.

January 22, 11 A. M. Operation and isolation of washed duodenal loop.

January 23, 3 P. M. Animal rather dull and lies on its side. Pulse pretty strong. Ether anesthesia and bleeding from carotid.

Autopsy.—Performed at once. The serous cavities and viscera are normal, except the duodenal loop. It contains 16 c.c. of slightly blood-stained soup-like material. The duodenal mucosa loop is quite intact and of a pinkish color. No hemorrhages.

Microscopical Examination.—The liver shows a little fatty degeneration, but is otherwise normal. The duodenal loop is quite normal except for hookworms buried in the mucosa.

The presence of a closed duodenal loop for twenty-four hours may not bring about any marked anatomical change. The viscera are normal and the loop usually shows an intact mucosa of pinkish color and contains a few cubic centimeters of mucoid material. At this time, however, the toxic material can be easily demonstrated in the contents and mucosa of the closed loop.

CLOSED DUODENAL LOOP. KILLED AFTER FORTY-FOUR HOURS.

Dog S–12.—February 6, 1912, 3 P. M. Operation with isolation of duodenal loop after careful washing.

February 7. Dog will not eat.

February 8. Vomits frothy mucus. Muscular tremors marked. 11 A. M. Ether anesthesia and bleeding from carotid. Autopsy at once. All the viscera are normal. The duodenum is greatly dilated and quite tense. The serous coat shows a few grains of fibrin, but the peritoneum elsewhere is clear. The loop contains 100 c.c. of turbid dark brown fluid. The mucosa of the loop, except for a little injection, is quite normal and intact. There are no ecchymoses or ulcers.

Microscopical Examination.—The mucosa of the duodenal loop is quite normal. There are no polymorphonuclear leucocytes seen anywhere. The liver and kidneys are likewise normal.

CLOSED DUODENAL LOOP. KYMOGRAPH. KILLED AFTER FORTY-FOUR HOURS.

Dog S–9.—Large mongrel; weight, 22 pounds.

February 3, 4 P. M. Operation with isolation of closed duodenal loop after careful washing.

February 4. Dog quiet and refuses food.

February 5, 11 A. M. Dog sick. Ether anesthesia and kymograph record from carotid. Blood pressure 100 to 110 mm. of mercury, with almost normal pulsation. Dog killed by bleeding.

Autopsy.—Performed at once. The viscera are negative, except the duodenal loop, which contains only 3 to 4 c.c. of concentrated dark colored material of buttery consistence. The mucosa is intact.

Microscopical Examination.—Liver: the cells show some fatty degeneration of a moderate grade and a little increase in cell pigment. The duodenal loop mucosa is normal.

CLOSED DUODENAL LOOP. KYMOGRAPH. KILLED AFTER FORTY-EIGHT HOURS.

Dog S–38.—Mongrel, male; weight, 17 pounds.

April 16, 3 P. M. Operation and isolation of duodenal loop after careful washing.

April 18. Dog is somewhat toxic and has vomited once. Rectal temperature, 39° C. 2 P. M. Ether anesthesia and kymographic tracings from carotid. The blood pressure was practically normal and the pulse regular. The dog was killed at the end of the observation. The thorax and viscera are negative. The peritoneal cavity is clean. The duodenal loop contains 20 to 30 c.c. of a strawberry colored thick fluid. The loop shows a diffuse pinkish mucosa, no ecchymoses, and no ulcers.

DUODENAL LOOP DRAINED. DEATH IN THIRTY DAYS.

Dog S–17.—Mongrel fox terrier, male; weight, 17 pounds.

February 16. Operation and isolation of duodenal loop, a rubber tube being sewn into one end of the duodenal loop which was fixed to the abdominal wall.

February 17 to 18. Animal appears toxic. The loop is rinsed out repeatedly by inserting a catheter.

February 19. The rubber tube has sloughed out from the end of the duodenum. Dog seems less toxic.

February 20. White mucus flakes escaping from the fistula. Dog seems slightly toxic. Duodenal washings contain a good deal of mucus.

February 24. Duodenal loop rinsed out thoroughly with catheter. Animal is improving and eats well.

March 9. Duodenal loop rinsed out thoroughly. Dog has lost a little weight but seems strong.

March 17. Dog is sick with distemper and killed with ether.

Autopsy.—Performed at once. The heart is normal. The lungs show a few patches of bronchopneumonia. The pancreas, liver, and kidneys are normal. The stomach shows a large gastro-enterostomy wound and no ulceration. The intestines are normal except for adhesions about the site of the duodenal loop. The drained loop is quite empty. Its mucosa is pale and normal.

Microscopical Examination.—The liver and kidneys are normal. The mucosa of the drained loop is normal in all respects.

DUODENAL LOOP DRAINED. DEATH IN ELEVEN DAYS.

Dog S–35.—Large mongrel collie.

March 11, 4 P. M. Operation with isolation and drainage of the duodenal loop. A rubber tube was fixed in the end of the duodenal loop.

March 12. The duodenal loop was washed out with a catheter. The washing is tinged with blood and contains much mucus. Dog not toxic.

March 13. Duodenal loop washed again.

March 14. Drainage tube has sloughed away. Dog fairly well except for a little vomiting.

March 17. Dog seems normal.

March 22. Dog in poor condition; has lost weight; some digestion of skin of abdomen. Ether anesthesia, and bled from carotid.

Autopsy.—Performed at once. All the viscera are normal. The loop of jejunum just below the gastro-enterostomy wound had opened into the abdominal wound discharging gastric and pancreatic juices, explaining the skin digestion and rapid loss of weight. The duodenal loop contains about 2 c.c. of slimy mucus. The mucosa is intact, but slightly pinkish. No ulcers and no submucous hemorrhages.

DUODENAL LOOP DRAINED. DEATH IN FIVE DAYS.

Dog S–49.—Strong mongrel bull, male; weight, 16 pounds.

May 11, 2 P. M. Operation and isolation with drainage of duodenal loop in the usual way.

May 12. Dog toxic. Duodenal loop washed out carefully.

May 13. Dog active and shows no signs of intoxication.

May 14. Duodenal loop washed out. Dog toxic and refuses food.

May 15. Wound breaking down. Duodenal loop washed out. Dog refuses food.

May 16. Dog is very cold and toxic and has lost considerable weight. Killed with ether and bleeding from carotid. Viscera in general are negative. The peritoneal cavity is clear except for firm adhesions about the drained loop. The stomach is full of bile-stained fluid. The small intestine is normal.

Duodenal loop is empty and collapsed. Its mucosa is pinkish but intact.

DUODENAL LOOP DRAINED. DEATH IN FIVE DAYS.

Dog S–46.—Fox terrier, male; weight, 15 pounds.

May 1, 3 P. M. Operation with isolation and drainage of duodenal loop as usual.

May 2, 11 A. M. Dog toxic. The duodenal loop was washed out by means of a catheter; the washings contained blood and mucus. They resemble closely the material found in some of the closed loops.

May 3. Duodenal loop again washed out thoroughly. Dog improving.

May 4. Duodenal loop washed out thoroughly; washings contain mucus.

May 6. Found dead in cage.

Autopsy.—Performed at once. The heart, lungs, and serous surfaces are normal. The spleen, pancreas, and liver are normal. The stomach contains brownish fluid much like that found in intestinal obstruction. The small intestine shows a pink and purple mottled mucosa like that found in some of the dogs poisoned by injection of toxic duodenal contents. The duodenal loop contains a few cubic centimeters of thick buttery material. The mucosa is quite intact and of a pinkish color.

Dogs with drained duodenal loops may live for months, and the first animal of this series lived for one month and died of distemper and bronchopneumonia. The second dog was in good condition until the jejunum opened into the abdominal wound.

The last two cases, however, are so interesting that they will be referred to later. Several other dogs showed an identical picture, and the reason for the intoxication is obscure. The drainage appeared to be perfect, and daily washings of the loop seemed to insure this, yet the dogs died after signs of intoxication similar to the closed-loop dogs. The mucosa of the drained loop dog shows the presence of a toxic substance (dog S–45[1]), and absorption obviously takes place from the epithelium and not the lumen which is empty.

DUODENAL LOOP EXCISED. KILLED AFTER FIFTY-THREE DAYS.

Dog S–15.—Old mongrel, male; weight, 20 pounds.

February 10. Operation and excision of the portion of the duodenum commonly isolated in the duodenal loop.

February 12 to 15. Quiet but eats. Some diarrhea.

February 18 to March 10. Dog seems wholly normal.

April 3. There has been trifling emaciation, but the animal is strong and in good health. Ether and bleeding from the carotid.

Autopsy.—Performed at once. The heart, lungs, and thorax are quite normal. The spleen is small and fibrous. The liver is pale, but otherwise normal. The kidneys, pancreas, and intestinal tract are normal. The area of operation is perfectly normal; gastro-enterostomy is functioning perfectly. There are no ulcers in the intestinal tract.

Microscopical Examination.—The kidneys and liver are normal. The spleen is of senile type. The duodenum and jejunum show a perfectly normal mucosa.

The experiments so far reported show clearly that this particular portion of the duodenum is not essential to life and that drainage of a duodenal loop under certain conditions may be perfectly compatible with life for months. Complete closure of this duodenal loop is always associated with severe intoxication and is followed by death in one to three days with a characteristic picture much like that of volvulus in human cases. There seems to be no escape from the assumption that some toxin is being formed in the closed duodenal loop and absorbed from it.

DUODENAL LOOP FLUID INTRAVENOUSLY. DEATH IN FOUR HOURS.

Dog S–37.—Mongrel, male; weight, 13.5 pounds.

April 13, 5 P. M. Ether anesthesia and kymograph record from carotid. Duodenal loop fluid from dog S–30 (closed duodenal loop; killed after forty-eight hours). Duodenal loop flaccid; contained 5 to 7 c.c. of strawberry colored thick fluid. This material was washed into a beaker with distilled water and allowed

[1] Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1913, xvii, 307.

to undergo autolysis at 38° C. under toluol and chloroform for a period of ten days. The supernatant fluid was poured off, heated to 60° C. for thirty minutes, centrifuged, and filtered. 40 c.c. of the clear broth-like fluid obtained were injected intravenously in small divided doses; an initial drop in blood pressure from the normal 180 mm. of mercury to 140 mm. occurred with rapid return to normal. During ten minutes following the last injections, a slow fall in blood pressure began, reaching 110 mm., and at the end of forty minutes, 65 mm. of mercury. During this fall there was considerable slowing of the pulse rate and variation in force and volume of heart beat. 5.45 P. M. Dog removed from kymograph; appears to be greatly shocked. 9 P. M. Dog found dead but quite warm. Abundant bloody mucus and fluid feces were passed, and blood-stained feces are oozing from the rectum.

Autopsy.—Performed at once. Blood removed from the heart shows no sign of clotting in thirty minutes; a flabby clot in twelve hours; and a firm clot in forty-eight hours. This is due to a great excess of antithrombin. The blood after centrifugalization shows only one fourth to one fifth plasma by volume, the normal plasma volume being one half of the whole blood. This condition results from great concentration of the plasma and loss of fluid from the blood. The heart, lungs, and kidneys are normal. The liver and spleen are greatly congested. The stomach contains a little fluid and shows a pink and red mottled mucosa over its cardiac portion. The pyloric portion is pale. The duodenum beginning sharply 1 cm. below the pylorus shows a swollen, deep purple, velvety mucosa (figure 1). The mucosa is coated with thick, creamy mucus which is loosely adherent. On removing this one sees the engorged villi as tiny red specks. The jejunum shows the same condition. The ileum shows a deep red mucosa fading gradually to the region of the ileocecal valve. The large intestine shows the same deep red mucosa and contains large amounts of blood-tinged fluid.

DUODENAL LOOP FLUID INTRAVENOUSLY. DEATH IN THREE HOURS.

Dog S-32.—Strong fox terrier, male; weight, 15 pounds.

March 28, 4 P. M. Ether anesthesia and kymograph record from carotid. Intravenous injection of duodenal loop fluid from dog S-21 (closed duodenal loop; killed after forty-four hours). The duodenal loop contained 5 c.c. of thick, dark paste which was washed into a beaker with distilled water and diluted to 60 c.c. with salt solution. The material was mixed thoroughly and allowed to stand at room temperature for four hours, was then heated at 60° C. for twenty minutes, centrifuged, and filtered through a Gooch crucible, centrifuged again, and the supernatant fluid used. The resulting opalescent broth-like fluid was injected in small doses intravenously. Blood pressure at the beginning, 190 mm. of mercury. 20 c.c. of duodenal loop fluid caused a prolonged drop in blood pressure to 80 mm. of mercury after a period of five minutes. Repeated small injections brought the blood pressure down to 60 mm. during the next twenty minutes. At the end of forty minutes the blood pressure was 100 mm. There was moderate slowing of the heart beat during this period of low blood pressure. 4.45 P. M. Dog removed from kymograph. 6.50 P. M. Cold extremities; death. Eyes show widely dilated pupils.

Autopsy.—Performed at once. Blood removed from the heart was fluid, and

there was no clot for thirty minutes. The next day the blood showed flabby clots. The lungs are normal. The spleen, liver, and kidneys show greatly engorged vessels. The mesenteric vessels in the abdominal cavity are much dilated and quite conspicuous. The stomach contains a little bile-stained mucus. The cardiac portion is reddish, the mucosa pinkish, while the pyloric portion is quite pale. There are no hemorrhages.

The duodenum beginning sharply 1 cm. below the pylorus shows a deep purple velvety mucosa. There is a thick, loosely adherent coat of mucus all over the surface. The villi stand out conspicuously as tiny bright red specks. The upper half of the small intestine shows a similar condition and the last foot of the ileum a pinkish mucosa. The large intestine is slightly reddened in its lower portion.

Microscopical Examination.—Duodenum: the villi show enormous engorgement of all their vessels. The deeper parts of the mucosa show only a trifling congestion, and the surface is coated with a thick layer of mucus. The epithelium appears normal. There is a trifling edema of the mucosa of the stomach. No acute inflammatory exudate is present. The kidneys and spleen, and the liver especially, show great congestion.

DUODENAL LOOP FLUID INTRAVENOUSLY. DEATH IN FOUR HOURS.

Dog S–71.—Small pup; weight, 10 pounds.

June 21 and 24. Dog injected with extracts of normal intestinal mucosa and the alcoholic extracts of duodenal fluid with negative result.

July 8, 5.30 P. M. Dog in excellent condition; weight, 11 pounds. Ether anesthesia and kymograph record from carotid. Intravenous injection of duodenal loop fluid from dog S–69 (closed duodenal loop; death after two and one half days). Duodenal loop fluid, autolysis for three days at 38° C. under toluol and chloroform. Heated at 60° C., centrifuged, and filtered. 55 c.c. of filtrate were injected slowly. Blood pressure before injection, 145 mm. of mercury; after injection, 70 mm.; and at the end of twenty minutes, 80 mm. There was marked slowing of the pulse during this period of low blood pressure. Feces were passed at the end of fifteen minutes following the injection. Muscular twitchings occurred.

9.30 P. M. Found dead.

Autopsy.—Performed next morning. The viscera are negative, except the intestinal tract. The cardiac portion of the stomach shows some congestion. The small intestine is greatly relaxed and contains large amounts of thin, blood-stained fluid. The mucosa beginning just below the pylorus is of a dull pink or red with considerable post-mortem digestion.

CLOSED DUODENAL LOOP FLUID INTRAVENOUSLY. ADRENALIN.

Dog S–27.—Fox terrier, male; weight, 12 pounds.

June 15, 12 M. Kymograph records from carotid. Duodenal fluid from dog S–53 (closed duodenal loop; death after sixty-six hours). The duodenal loop contained a little pasty material which was scraped out of the loop together with the mucosa, diluted with a little water, and allowed to undergo autolysis under toluol at 38° C. (May 16). Autolysis for one month at 38° C. Fluid heated to 60° C. for one hour, centrifuged, and filtered. 17 c.c. of filtrate given intrave-

nously. This caused a fall from 130 mm. of mercury to 60 mm., with a rapid rise above normal to 160 and return to normal. The second fraction of duodenal loop fluid gave a similar result, followed by a slow secondary fall during the next five to ten minutes, reaching a level of 80 mm. of mercury. A small amount of adrenalin, 2 c.c. of a 1:10,000 solution, gave the usual result with a rise to 240 mm. of mercury. At the end of one hour the intestine was opened and the mucosa of the jejunum presented a deep red velvety appearance. This did not bleach on giving adrenalin intravenously nor on adding a solution of adrenalin directly to the mucosa. The dog was killed at the end of one and one half hours.

Autopsy.—Performed at once. The usual congested intestinal mucosa was seen, beginning just below the pyloric ring. The viscera were negative, except for congestion.

The preceding experiments show that the material which accumulates in a closed duodenal loop is highly toxic when introduced intravenously. This material in the loop may be thin and soup-like or thick and pasty, but in all cases the toxic material can be demonstrated. Only a few cubic centimeters of this material (dogs S–37 and 32) may be diluted, incubated at 38° C. under toluol for days, then heated at 60° C. for thirty minutes, and filtered without removing or destroying the toxic substance. Intravenous injection of this broth-like substance will cause an initial drop in blood pressure followed by a rise to normal and a prolonged secondary fall to one half or one third of normal. The heart beat is slowed and may become irregular at this time. The dog is then in a condition of severe shock and the temperature falls; the pulse is scarcely palpable and muscular tremors are conspicuous. Vomiting and profuse diarrhea occur in the fatal cases and blood streaks appear in the watery stools. Respiration becomes slow and very deep some time before death. In these dogs it is to be remembered that a large amount of toxic material was given even though a small amount of concentrated duodenal loop material was used. It is probable that at least double the lethal dose was given in these first experiments, and it is possible to give a sufficient amount of poison to kill a normal dog in twelve to twenty hours. The anatomical findings in the dogs poisoned less acutely may not be as striking, but in general are quite similar. The acutely poisoned dogs here described present characteristic anatomical lesions. The blood remains fluid much longer than is normal, and clots form slowly, but can be accelerated by tissue extracts, showing the pres-

ence of an excess of antithrombin. The blood plasma becomes concentrated and the dried weight of the blood is above normal, as would be expected when the great loss of fluids by the alimentary tract is recalled. The thorax, heart, and lungs are normal. The liver, spleen, and mesenteric vessels show great engorgement, and the liver may be very tense and elastic. The gastro-intestinal tract shows the most striking changes (figure 1). The stomach contains bile-stained fluid, and the cardiac portion shows a moderate congestion of the mucosa, but the pyloric end is quite pale. About one centimeter below the pylorus there is a sharp line of demarcation separating the pale mucosa from the deep purple velvety mucosa of the duodenum. The duodenum is relaxed, usually contains fluid, and its engorged mucosa is coated with thick mucus. The intestinal contents are blood-tinged and rich in mucus. The jejunum presents a similar picture, but the intensity of the color usually fades to pink in the lower ileum. The large intestine shows congestion of its mucosa and ecchymoses are not infrequent.

Microscopical Examination.—There is great engorgement of all capillaries in the villi and no other change except occasionally some escape of red blood cells into the tissue.

Adrenalin given during the period of low blood pressure causes the usual reaction, but does not effect any bleaching of the intestinal mucosa whether given intravenously or applied directly to the surface.

Secretin can be prepared from the jejunum of a closed-loop dog and acts in the usual manner, but the material from the closed duodenal loop in the same dog will cause no pancreatic reaction when given intravenously.

DUODENAL LOOP FLUID INTRAVENOUSLY. DEATH IN FOUR HOURS. SECRETIN ABSENT.

Dog S–13.—Strong adult male; weight, 24 pounds.

Kymograph experiment with tracings from the carotid, and injection of material into the jugular vein. The cannula was placed in the pancreatic duct and the flow was recorded by a Marey tambour. Anesthesia with ether and 0.25 of a grain of morphia. Duodenal fluid obtained from dog S–12 (closed duodenal loop; killed after forty-four hours), centrifuged at high speed, and filtered through a thick Gooch crucible. Fluid very faintly acid to litmus. Duodenal fluid (10 c.c.) given intravenously caused a slight drop in blood pressure, but

there was no flow from the pancreatic duct. Blood pressure before injection, 140 mm.; five minutes after injection, 130 mm. of mercury.

Secretin was made from the normal duodenal and upper jejunal mucosa (dog S–12), by the method of Bayliss and Starling. Secretin (10 c.c.) produced a great drop in blood pressure; namely, from 140 to 60 mm. of mercury. There was prompt and striking pancreatic secretion within about fifteen seconds, lasting several minutes, during which time the blood pressure came back nearly to normal. Duodenal loop fluid (8 c.c.) was again injected, followed by a slight drop in blood pressure, but no pancreatic flow. After an interval of a few moments a third injection of acidified duodenal loop fluid (10 c.c.) was given. This caused no influence upon the pancreatic flow, but caused a slow drop in blood pressure, reaching 60 mm. of mercury. There was some slowing in the pulse rate. Blood removed at the end of the kymograph observation clotted very slowly, requiring about four times the normal time to clot. 1 P. M. Dog removed from kymograph and abdomen closed. 2.30 P. M. Slow deep respiration and very weak pulse. 4 P. M. Death occurred in the condition of shock with slow deep respiration and cold extremities.

Autopsy.—Performed at once. Blood taken from the heart required two hours for the formation of soft clots. The heart is negative. The lungs are slightly dilated. The spleen, liver, and kidneys show congestion. The stomach contains a little blood-tinged mucus, and its mucosa is pale. The duodenum beginning 1 cm. below the pyloric ring shows a deep purple and velvety mucosa coated with mucus. This picture is most intense throughout the duodenal loop, but there is a deep red mucosa throughout the jejunum fading to a pink color in the ileum. The large intestine shows a deep red mucosa. The intestine contains fluid which is slightly blood-stained.

Microscopical Examination.—The kidneys are practically normal. The liver shows a good deal of congestion. The gastric mucosa shows a little congestion, but the villi of the duodenum show enormous engorgement of all their capillaries. There is no inflammatory reaction, simply great widening of the capillaries in the ends of the villi and engorgement with red blood cells. No thrombi are seen, and no escape of blood into the tissue.

DUODENAL LOOP FLUID INTRAPORTALLY. DEATH IN THREE HOURS.

Dog S–40.—Fox terrier, male; weight, 13 pounds.

April 20, 11.30 A. M. Kymograph records taken from the carotid. Ether anesthesia and abdominal incision with aseptic precautions. A cannula was tied in a branch of the portal vein, and duodenal loop fluid from dog S–30 was given at intervals; the total amount was 20 c.c. The same fluid was used in dog S–37 with fatal intoxication. There was a fairly rapid drop in blood pressure from the normal 155 mm. of mercury to 60 mm. Then followed a slow return to about 110 mm. during the next five or ten minutes. The curve was similar to that produced after intravenous injection. A marked secondary fall occurred during the next twenty minutes to 60 mm. At the end of thirty minutes a loop of the jejunum was opened and showed a deep pink mucosa which became more and more engorged during the following fifteen minutes. There was a marked secretion of mucus and clear fluid. At the end of the observation the mucosa had the characteristic purple appearance found at autopsy. The

mesenteric vessels were very conspicuous. The intestine and abdomen were closed in the usual way. 12.30 P. M. Blood drawn from the jugular vein. Dry weight 24.1 per cent. in two parallel determinations. Rectal temperature 34.5° C., in spite of artificial heat applied by an electric pad. 1 P. M. Slow, deep respiration. Involuntary passage of thin, yellow, faintly blood-streaked stools. 2.30 P. M. Death. Muscular tremors were conspicuous, and the pupils dilated.

Autopsy.—Performed at once. The heart was found beating slowly and continued to beat many minutes after opening the thorax. The lungs were normal. The spleen, liver, and kidneys were greatly engorged with blood. Blood obtained from the heart did not clot in thirty minutes. Flabby clots formed in eighteen hours, and solid clots in forty-eight hours. Dried weight of blood, 24.8 per cent. and 24.9 per cent. in parallel determinations. The stomach shows a pale mucosa about the pylorus and a deep pink in the cardia. The duodenum one centimeter below the pylorus shows a deep purple velvety mucosa covered with thick adherent mucus. The jejunum shows the same picture. The ileum shows a deep red mucosa and contains large amounts of thin, blood-stained fluid. Some tiny specks of hemorrhage are seen in the submucosa in the ileum.

DUODENAL LOOP FLUID INTRAPERITONEALLY. DEATH IN TWELVE HOURS.

Dog S–52.—Small mongrel, female.

May 15, 12 M. Ether anesthesia and kymograph tracings from carotid. Blood pressure at start, 160 mm. of mercury. Duodenal loop fluid from dog S–11 (closed duodenal loop; killed after twenty-four hours). The closed-loop (dog S–11) contained only 5 c.c. of a pinkish, thin soup-like material which was washed out into a beaker with distilled water. The mucosa of the loop was scraped off and added to the loop fluid with a little toluol and chloroform in a closed flask. After autolysis at 38° C. for twelve days the fluid was heated at 60° C. for one half hour, centrifuged, and filtered. The total filtrate (60 c.c.) was injected intraperitoneally. 1 P. M. Blood pressure was 120 mm. of mercury. Dog was removed from the kymograph. 2.30 P. M. Dog is much shocked. Pulse is very feeble. No diarrhea, some vomitus, and salivation. Rectal temperature, 37° C. 4.30 P. M. Repeated vomiting; then watery diarrhea. Rectal temperature, 39° C. 5.30 P. M. Diarrhea continued. Pulse weak and scarcely palpable. Respirations slow and deep. Pupils widely dilated. 8.30 P. M. Rectal temperature, 37° C. Diarrhea continued. May 6, 10 A. M. Found dead and cold.

Autopsy.—The heart is dilated with flabby clots. The lungs are negative. The spleen and liver are deeply congested. The stomach contains watery bile-stained fluid. The mucosa over the cardiac portion is reddish and mottled. The pylorus is pale. The duodenum shows a mottled mucosa, purplish areas 1 to 3 cm. in diameter being thickly scattered over the reddish mucosa. The same condition is found in the jejunum. The ileum is relatively pale. The large intestine shows considerable injection of the mucosa. The intestine contains thin, fluid, blood-stained material. This picture is almost identical with the findings in some of the dogs dying with closed duodenal loops. In both instances we may assume a relatively slow absorption of the toxic substance (compare dog S–53).

DUODENAL LOOP FLUID SUBCUTANEOUSLY. DEATH IN EIGHTEEN HOURS.

Dog O–8.—Small, adult fox terrier, female; weight, 8 pounds.

November 1, 10.30 A M. Ether anesthesia and subcutaneous injection of duodenal loop fluid obtained from dog S–90 (closed duodenal loop; immunized; killed after four days). The closed loop contained about 10 c.c. of thick pasty material which was diluted with about 100 c.c. of distilled water; chloroform and toluol were added and the mixture was allowed to undergo autolysis for three months. The supernatant fluid was heated at 60° C. for thirty minutes, centrifuged, and filtered. The total filtrate (100 c.c.) was injected subcutaneously over the abdomen and thorax and the areas were massaged. Ether was stopped at the end of fifteen minutes. 11 A. M. Pulse is weak and just palpable. 11.30 A. M. Pulse slightly stronger; respiration rather slow and deep. 5.00 P. M. Dog walks about; one semifluid stool; appears quite sick; ecchymoses numerous over areas of injection.

November 2, 9 A. M. Dog found dead and cold.

Autopsy.—The subcutaneous tissue at the sites of injection is edematous and dotted with ecchymoses. The serous surfaces are all normal. The heart contains no clots. The lungs are pink and normal. The spleen and liver are moderately congested. The stomach contains a little bile-stained fluid and shows a pinkish mucosa. The duodenum contains much fluid and mucus. Its mucosa is swollen and mottled red and purple. The jejunum and upper ileum show the same picture. The lower ileum is normal. The large intestine shows red splotches over its mucosa and apparently some hemorrhage into the submucosa.

The three preceding experiments show that the toxic substance causes the characteristic reaction in a normal dog, whether introduced into the portal vein, peritoneal cavity, or subcutaneous tissues. Intraportal injection gives a reaction practically identical with the intravenous. When the duodenal fluid is introduced subcutaneously or intraperitoneally the absorption is relatively slow, and other things being equal, the dogs survive longer.

The same symptoms, however, are noted, and autopsy reveals the same general changes, although less intense in degree. The intestinal tract excretes a large amount of fluid and suffers much congestion of its mucosa, especially in the duodenum. It will be seen that this reaction and the autopsy findings may be practically duplicated in dogs that have died after a closed duodenal loop has been established (compare dog S–53). In both cases we may assume a relatively slow absorption of the toxic fraction of the duodenal loop fluid, in the one case by the subcutaneous tissue, and in the other by the duodenal loop mucosa.

SUMMARY.

Closed duodenal loops may be made in dogs by ligatures placed

just below the pancreatic duct and just beyond the duodenojejunal junction, together with a posterior gastro-enterostomy.

These closed duodenal loop dogs die with symptoms like those of patients suffering from volvulus or high intestinal obstruction. This duodenal loop may simulate closely a volvulus in which there has been no vascular disturbance.

Dogs with closed duodenal loops which have been washed out carefully survive a little longer on the average than animals with unwashed loops. The duration of life in the first instance is one to three days, with an average of about forty-eight hours.

The dogs usually lose considerable fluid by vomiting and diarrhea. A weak pulse, low blood pressure and temperature are usually conspicuous in the last stages.

Autopsy shows more or less splanchnic congestion which may be most marked in the mucosa of the upper small intestine. The peritoneum is usually clear and the closed loop may be distended with thin fluid, or collapsed, and contain only a small amount of pasty brown material. The mucosa of the loop may show ulceration and even perforation, but in the majority of cases it is intact and exhibits only a moderate congestion.

Simple intestinal obstruction added to a closed duodenal loop does not modify the result in any manner, but it may hasten the fatal outcome.

The liver plays no essential rôle as a protective agent against this poison, for a dog with an Eck fistula may live three days with a closed loop. A normal dog reacts to intraportal injection and to intravenous injection of the toxic substance in an identical manner.

Drainage of this loop under certain conditions may not interfere with the general health over a period of weeks or months.

Excision of the part of the duodenum included in this loop causes no disturbance.

The material from the closed duodenal loops contains no bile, pancreatic juice, gastric juice, or split products from the food. It can be formed in no other way than by the activity of the intestinal mucosa and the growth of the intestinal bacteria.

This material after dilution, autolysis, sterilization, and filtration produces a characteristic effect when introduced intravenously. When in toxic doses it causes a profound drop in blood pressure,

general collapse, drop in temperature, salivation, vomiting, and profuse diarrhea, which is often blood-stained.

Splanchnic congestion is the conspicuous feature at autopsy and shows especially in the villi of the duodenal and jejunal mucosæ.

Adrenalin, during this period of low blood pressure and splanchnic congestion, will cause the usual reaction when given intravenously, but applied locally or given intravenously it causes no bleaching of the engorged intestinal mucosa.

Secretin is not found in the duodenal loop fluid, and the loop material does not influence the pancreatic secretion.

Intraportal injection of the toxic material gives a reaction similar to intravenous injection.

Intraperitoneal and subcutaneous injections produce a relatively slow reaction which closely resembles the picture seen in the closed duodenal loop dog. In both cases there is a relatively slow absorption, but the splanchnic congestion and other findings, though less intense, are present in both groups.

There seems, therefore, to be no escape from the conclusion that a poisonous substance is formed in this closed duodenal loop which is absorbed from it and causes intoxication and death. Injection of this toxic substance into a normal dog gives intoxication and a reaction more intense but similar to that developing in a closed-loop dog.

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EXPLANATION OF PLATE 55.

FIG. 1. Stomach and duodenum of a dog acutely poisoned with fluid obtained from a closed duodenal loop (dog S–37). The purplish red color is due to extreme engorgement of the villi in the duodenum. The mucosa is covered with fluid and bile-stained mucus.

FIG. 1. (Whipple, Stone and Bernheim: Intestinal Obstruction.)

INTESTINAL OBSTRUCTION.

II. A Study of a Toxic Substance Produced by the Mucosa of Closed Duodenal Loops.*

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In the preceding paper we have shown that dogs with closed duodenal loops die in a characteristic manner, due to the absorption of some poison from this loop. In human beings death from volvulus or high intestinal obstruction may occur under symptoms which are practically identical with those observed in these dogs. It seems fair to assume that a similar intoxication exists in human cases, and that a study of the condition in dogs may throw some light on the obscure points attending intestinal obstruction in man. A study of intestinal obstruction in man is beset with many difficulties arising from the complexity of the phenomena and difficulty of obtaining cases in a favorable condition for investigation. Nevertheless, we hope to make a report upon human cases in the near future.

In this paper we shall report a series of experiments showing that a toxic substance is produced by the intestinal mucosa and can be demonstrated in it, and that the poison will not be formed in closed duodenal loops when the mucosa has been destroyed by chemical means. Moreover, no such poison can be demonstrated in the normal intestinal mucosa.

The blood taken from a dog with a duodenal loop is not toxic to a normal dog when given intravenously. It makes no difference whether the blood removed from the heart after death is taken during the first twenty-four hours after the production of a closed duodenal loop or after forty-eight hours. In neither instance does the injection of 100 to 300 cubic centimeters of the defibrinated blood or serum in a normal dog cause any intoxication or change in blood pressure.

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This observation agrees with that of McLean and Andries, and yet we do not believe that one is justified in assuming that no toxic substance has been absorbed into the blood stream. Our third experiment given below shows the fallacy of such reasoning. In the experiment a normal dog (S–10) was fatally poisoned with the fluid obtained from a closed duodenal loop. Blood obtained from this dog at the time of death was defibrinated, centrifuged, and the clear serum (80 cubic centimeters) injected intravenously into a normal dog. The normal dog (S–11) showed no change in blood pressure and no intoxication following the injection. After an interval of one month this same dog (S–11) was given a second intravenous injection of defibrinated blood (120 cubic centimeters) obtained from a dog (S–22) fatally poisoned with duodenal loop fluid. This second injection, like the first, was absolutely without toxic effect.

The experiment shows that in two hours or less a toxic substance may be removed from the blood stream. A known toxic substance was introduced directly into the blood stream and two hours later at autopsy the blood of this animal was quite free from the toxic substance, as was shown by intravenous injection into a normal dog. One should not be surprised, therefore, to learn that the blood of a closed-loop dog is non-toxic, for the absorption of the toxic substance within the closed loop must be relatively a slow process. The explanation of the fact is not clear, but it is probable that the substance is rapidly fixed by the tissues or is in part destroyed. However, more work is required to elucidate this point.

The experiments show also that the blood of the poisoned dogs at the time of observation contained no foreign protein or substance capable of giving rise to anaphylactic shock. It has been noted in a previous communication that the phenomena of anaphylaxis in the dog, such as low blood pressure and temperature, blood incoagulability, elimination of fluid into the alimentary tract, and great splanchnic congestion, are similar to those produced by an intravenous injection of the material from a closed duodenal loop.

EXPERIMENTAL OBSERVATIONS.

THE BLOOD FROM A CLOSED DUODENAL LOOP DOG IS NON-TOXIC.

Dog C–23.—Black mongrel, male; weight, 22 pounds.

January 20, 4 P. M. The carotid artery was connected with the kymograph. Dog was first bled 100 c.c. from the jugular vein, which caused some drop in blood pressure. This was followed by a rapid intravenous injection of 200 c.c. of defibrinated blood from dog S–4 (closed duodenal loop dog; killed after twenty-four hours). The injection caused no reaction except a slight rise above normal in blood pressure. The second injection of 100 c.c. caused a rise in venous pressure, but no marked change in arterial pressure. Dog was removed from the kymograph and seemed normal when recovering from the ether.

January 21. Dog is lively and eats well.

February 27. Dog is in excellent condition; weight, 25 pounds. The other carotid was attached to the kymograph and a record of blood pressure taken. 100 c.c. of defibrinated heart blood from dog S–20 (closed duodenal loop dog; death in forty-eight hours) was injected rapidly intravenously without affecting the blood pressure in the slightest way.

This experiment shows the absence of the toxic material from the circulating blood and of foreign protein capable of causing anaphylactic shock.

Dog S–3.—Small mongrel, female.

January 11, 3 P. M. A kymographic record was obtained from the carotid while 130 c.c. of blood from dog S–2 (closed duodenal loop dog; killed after twenty-six hours) was injected intravenously. No change in the blood pressure occurred during a period of thirty minutes. The injection was followed by a second injection of 22 c.c. of the duodenal contents from dog S–2 which caused a profound drop in blood pressure. 6.30 P. M. Animal is deeply shocked. Respirations are slow and deep. Vomiting and diarrhea. The pupils are dilated.

January 12, A. M. Dog found dead.

Autopsy.—Performed at once. The serous surfaces are negative. The blood has not clotted. The heart is negative. The lungs are rather voluminous and show some congestion posteriorly. The spleen is soft and congested. The liver is greatly congested. The pancreas and kidneys are normal. The stomach is full of food. The mucosa is normal and pale.

The duodenum shows a deep purple, velvety mucosa beginning about 1 cm. below the pylorus. It contains bloody fluid. This condition occurs throughout the jejunum but fades to a paler color in the ileum. The duodenal contents were toxic and gave the typical picture outlined above, while the blood from the same animal was non-toxic.

THE BLOOD FROM A DOG POISONED WITH DUODENAL LOOP FLUID IS NON-TOXIC.

Dog S–11.—Brindle pup, male; weight, 17½ pounds.

February 7, 12 M. Kymographic observation from the carotid and intravenous injection of heart blood from dog S–10 (poisoned with duodenal fluid; death in two hours). 80 c.c. of blood serum gave no reaction; dog was normal at the end of the experiment.

February 9. Dog is well.

March 5. Kymograph as above, and intravenous injection of heart blood from dog S–22 (poisoned with duodenal fluid intravenously; death in two and one half hours). 120 c.c. of blood injected rapidly caused a slight rise in blood

pressure, which returned to normal in a few minutes. Dog is normal at the end of the experiment.

March 11. Dog is well.

This experiment shows how rapidly the toxic material obtained from the duodenal loop may disappear from the circulating blood. Presumably it is fixed by the tissue cells or partly destroyed. This experiment agrees with the preceding ones in every respect, but it is more convincing, as a known toxic substance had been introduced into the blood stream.

CLOSED DUODENAL LOOP MUCOSA. DEATH IN SIXTEEN HOURS.

Dog S–33.—Young fox terrier, female; weight, 16 pounds.

March 29, 4 P. M. Ether anesthesia and kymographic observation as usual. Mucosa of the duodenal loop was obtained from dog S–21 (closed duodenal loop dog; killed after forty-three hours). The closed loop contained only a small amount of thick, pasty, brown material which was washed off carefully. The mucosa which had been washed free from the fluid was then scraped from the intestinal wall, mixed with 0.9 per cent. salt solution, and put in the thermostat for twenty-four hours. There was considerable bacterial activity with complete solution of the mucous membrane. The fluid was heated at 61° C. for twenty minutes, centrifuged, and filtered through a Gooch crucible. 62 c.c. of the filtrate were given intravenously in divided doses. The normal blood pressure was 150 mm. of mercury. Small amounts of this filtrate caused an initial fall in blood pressure with a slow return toward normal, reaching 115 mm. at the end of twenty minutes. Later injections gave the same reaction, with the general tendency to return to normal. At the end of forty minutes the blood pressure was 110 mm.; at the end of one hour it was the same. There was a definite slowing but no irregularity in the heart beats. 5.15 P. M. Dog was removed from the kymograph.

March 30, 9 A. M. Dog found dead and cold.

Autopsy.—Performed at once. The serous cavities are normal. The heart contains firm clots. The right lung is clear and the left lung shows bronchopneumonia, edema, and some hemorrhage throughout. This was explained by aspiration of some vomitus during the kymographic observation, the dog lying on this side and all the material being aspirated into that lung. The spleen and liver are considerably congested. The kidneys are rather pale. The stomach is negative. The duodenum shows the usual purplish, velvety mucosa and contains a good deal of fluid. The jejunum shows a pale pinkish mucosa fading to a normal ileum.

Microscopical Examination.—The duodenum shows considerable congestion of its upper portion and beginning post-mortem digestion of the villi. The lungs show an exudate of polymorphonuclear leucocytes, blood cells, and blood-stained granular material, and no fibrin. The thymus is greatly congested and small hemorrhages are present. The liver also is greatly congested, particularly in the centers of the lobules.

CLOSED DUODENAL LOOP MUCOSA IS TOXIC BUT NOT FATAL.

Dog O–13.—Adult terrier, male; weight, 9 pounds.

November 23, 10.45 A. M. Rectal temperature, 38.5° C. Ether anesthesia was given for a few minutes and fluid was injected into the jugular vein. This fluid was obtained from the loop mucosa of dog O–11 (closed duodenal and jejunal loop; death in three days). The closed loop (dog O–11) was distended with turbid gray fluid, and the mucosa was ulcerated in places. After opening and rinsing the loop, its mucosa was scraped off carefully, diluted with 0.9 per cent. salt solution, and put in the thermostat at 38° C. for autolysis with a little chloroform and toluol. Autolysis for six days. The fluid was heated at 60° C. for thirty minutes, centrifuged, and filtered, giving a clear, amber, broth-like fluid which was faintly alkaline to litmus. The total filtrate (90 c.c.) was given slowly with no apparent effect. 11 A. M. Temperature, 37° C. Dog is out of ether. 11 to 11.30 A. M. Dog is active and noisy, apparently quite normal. Pulse is strong and tension is normal. 11.45 A. M. Dog passed a soft stool and pulse shows some slowing. 12 M. Dog is quiet and looks sick and dull. The pulse is weak and slow. Dog passed soft stool and there is tenesmus. Respiration is slow and deep. 12.30 P. M. Dog has passed much fluid feces, but has not vomited. The pulse is weak and easily compressed. 1 P. M. Temperature, 38.3° C. The pulse is very weak and dog is gravely shocked; lies on side in semistupor. 2.30 P. M. Temperature, 39.9° C. Condition is the same. 4.45 P. M. Dog is very sick. Temperature, 39.4° C. 8 P. M. There has been no more diarrhea or vomiting. The pulse is very weak. Animal is dull and stupid.

November 24, 10 A. M. Dog is much improved and on the way to recovery. Temperature, 38.2° C.

Dog S–97.—Fox terrier, female; weight, 12½ pounds.

July 22, 10.30 A. M. Ether anesthesia and kymographic observation as usual. Intravenous injection of duodenal loop mucosa from dog S–70 (closed duodenal loop dog; immunized; death in four days). This loop contains a little fluid and its mucosa is intact. The loop mucosa was washed carefully, scraped off, and added to 0.9 per cent. salt solution with chloroform and toluol. Autolysis at 38° C. for seven days. The fluid was heated at 60° C. for forty-five minutes, centrifuged, and filtered. The broth-like filtrate, 30 c.c. in amount, was given intravenously. The blood pressure at the beginning of the experiment averaged 145 mm. of mercury. The intravenous injection of the fluid caused only a moderate drop in blood pressure with a fairly rapid return above normal to a pressure of 180 mm. of mercury. The pressure fell slowly during the next ten minutes to a level of 110 mm., at the end of the observation. 11.30 A. M. Dog removed from the kymograph. 3 P. M. Dog is greatly shocked. The pulse is weak. Marked muscular tremors. No diarrhea.

July 23. Dog has recovered and appears normal.

DRAINED DUODENAL LOOP MUCOSA IS TOXIC BUT NOT FATAL.

Dog S–45.—Mongrel pup, male; weight, 16 pounds.

May 1, 12 M. Ether anesthesia and kymographic observation. Drained duodenal loop mucosa was obtained from dog S–35 (death in eleven days). The mucosa was intact and pinkish, and the loop contained about 2 c.c. of slimy mucus. The mucosa was scraped off carefully and diluted with distilled

water; chloroform and toluol were added. Autolysis at 38° C. for five weeks. The supernatant fluid was centrifuged and the clear, odorless, broth-like fluid was used without heating. The total filtrate (40 c.c.) was given intravenously. The blood pressure at the beginning of the experiment was 130 mm. of mercury. The intravenous injection in divided doses caused a trifling drop in blood pressure to 100 mm., followed by a rise slightly above normal to 140 mm. During the next twenty-five minutes a slowly progressing drop began, reaching 50 mm. after forty-five minutes. At the end of sixty minutes there had been a slight recovery of blood pressure to 90 mm. There was slowing and irregularity of the heart beat at this time. 1 P. M. Dog removed from kymograph. 1.30 P. M. Dog is much shocked. Diarrhea is marked, and fluid feces are slightly blood-tinged. Vomitus is bile-stained. 2 P. M. Dog is weak but somewhat improved. Diarrhea is still present. The pulse is weak.

May 2. Dog is much improved.

May 3. Animal seems to have recovered.

May 4. Found dead.

Autopsy.—Performed at once. No adequate cause for death is revealed in spite of careful investigation. The organs were practically normal throughout.

Dog O–4.—Male pup; weight, 8 pounds.

October 26, 10 A. M. Ether anesthesia and intravenous injection. The mucosa was obtained from dog O–2 (drained duodenal loop dog; death in six days). The drained loop was not dilated and contained about 4 c.c. of buttery material. Its mucosa shows one small ulcer 6 mm. in diameter close to the edge of the abdominal wound. In general it is red or faint pink. The pasty material together with the mucosa of the loop was scraped off carefully into 0.9 per cent. salt solution; chloroform and toluol were added and the mixture was put away for incubation at 39° C. Autolysis for four days. The supernatant fluid, heated at 60° C. for thirty minutes, centrifuged, and filtered, gave a clear, broth-like, odorless fluid. The total filtrate (70 c.c.) was given intravenously, without kymographic records. Ten minutes after the injection the skin over the abdomen showed marked flushing with patches of urticaria. The pulse became very weak twenty minutes after the injection, and the flushing of the skin disappeared, but the patches of edema were still present. 11 A. M. Vomited much bile-stained fluid. 11.30 A. M. Vomiting continued, salivation marked, and semifluid feces passed. 3 P. M. Involuntary passage of fluid and feces. Dog is cool and greatly shocked. Pulse is weak. The skin is pale and the localized edema has disappeared. Etherized and killed. The thorax, heart, and lungs are normal. The liver and spleen show considerable engorgement. The duodenum shows only slight injection of the mucosa, and the small intestine is practically normal except for an excess of bile-stained fluid. It is probable that this animal would have recovered completely.

The five preceding experiments give clear evidence that a toxic substance can be isolated from the duodenal loop mucosa which may be quite normal in gross and under the microscope. This fact speaks strongly for the view that the poison is elaborated by the activity of the mucosa and not by the intestinal bacteria. The final

proof of the point is given below, where it is shown that destruction of the mucosa prevents the formation of the toxic substance.

The fact that the same toxic substance can be demonstrated in the mucosa of a drained, washed duodenal loop is of special interest. This may occur in spite of daily irrigations of the loop, thus insuring perfect drainage and no accumulation under tension. In this is explained the fact that many of the drained duodenal loop dogs, in spite of good drainage and irrigation, will die after five or six days under symptoms of intoxication, and at autopsy give evidence of a type of intoxication similar to that found in ordinary closed duodenal loop dogs.

From this it is concluded that absorption of the toxic substance may occur from a mucosa which seems normal except for trifling congestion. There can scarcely be any absorption from the lumen as all the material escapes or is washed out; and hence we must assume that the absorption takes place from the mucosa alone where surely this toxic substance is elaborated. We may go further and suppose that in the closed duodenal loops with intact mucosa the greater part of the absorption takes place from the mucosa and not from the material which collects in the lumen.

NORMAL INTESTINAL MUCOSA OF THE DUODENUM AND JEJUNUM ARE NON-TOXIC.

Dog S–71.—Small pup; weight, 10 pounds.

June 21, 12 M. Ether anesthesia and kymograph as usual. Normal intestinal mucosa from dog C–80, which weighed 30 pounds. Immediately after death the duodenum and about one foot of the upper jejunum were opened, washed out carefully, and the entire mucosa was scraped off and put away for autolization with 0.9 per cent. salt solution, toluol, and chloroform. Autolysis at 38° C. for eight days, and then heated at 60° C. for one hour, centrifuged, and filtered, giving an opalescent broth-like fluid. The entire filtrate (55 c.c.) given intravenously caused an initial drop in blood pressure from 110 mm. to 65 mm. of mercury, followed by a rapid return above normal to 170 mm. with slow return to normal. The second fraction caused much the same reaction with a drop followed by a return above normal. At the end of twenty minutes blood pressure was 110 mm., and the pulse was regular and normal. 2 P. M. No diarrhea. Dog has vomited a little water and mucus. The pulse is strong.

June 22. Dog is active and well.

NORMAL MUCOSA OF THE JEJUNUM IS NON-TOXIC.

Dog C–9.—Small female; weight, 12½ pounds.

March 11, 4 P. M. Ether anesthesia and kymographic observation. Jejunal mucosa was obtained from a normal dog. The intestine was opened and washed,

and the mucosa scraped off. Water, toluol, and chloroform were added. Autolysis for six days at 38° C. The supernatant fluid was centrifuged and filtered through a Gooch crucible. The clear filtrate (48 c.c.) was given intravenously. Blood pressure at the beginning of the experiment was 150 mm. of mercury. The fluid caused a drop in blood pressure to 110 mm. followed by a rapid return to normal. At the end of twenty minutes the blood pressure was 140 mm. The pulse was regular and normal. Dog showed no signs of intoxication and recovered rapidly.

NORMAL INTESTINAL MUCOSA IS NON-TOXIC.

Dog S–42.—Mongrel, male; weight, 11 pounds.

April 26, 4 P. M. Ether anesthesia and kymograph as usual. Intestinal mucosa was obtained from a normal dog. The entire small intestine was opened and washed. The mucosa was completely scraped off and put in a flask with about 100 c.c. of distilled water, chloroform, and toluol. Autolysis at 38° C. for six weeks. The supernatant fluid was centrifuged and filtered. The filtrate was an odorless, broth-like fluid, 85 c.c. in amount. This material given intravenously caused the usual drop in blood pressure from 120 to 75 mm. of mercury. There was a rapid return to normal after each injection. At the end of twenty-five minutes the blood pressure had returned to 120 mm. The dog was removed from the kymograph.

April 27. Dog is normal.

NORMAL MUCOSA OF THE ILEUM IS NON-TOXIC.

Dog C–23.—Mongrel, male; weight, 25 pounds.

March 11. Ether anesthesia and kymograph. Mucosa from the ileum of a normal dog was obtained after washing, and was put away as usual for autolysis at 38° C. for six days. Centrifuged and filtered through a Gooch crucible. The clear broth-like filtrate (43 c.c.) was given intravenously. This fluid had a marked but transient depressor action. Blood pressure at the beginning of the experiment was 180 mm.; it fell to 80 with rapid recovery to normal. At the end of twenty minutes the blood pressure was 170 mm. The dog was removed from the kymograph after thirty-five minutes. It appeared normal.

March 12. The dog is perfectly well. There has been no vomiting or diarrhea.

INTESTINAL MUCOSA WITH PUTREFACTION IS NON-TOXIC.

Dog S–30.—Mongrel terrier, male; weight, 20 pounds.

March 27, 11 A. M. Ether anesthesia and kymograph as usual. Intestinal mucosa from the entire small intestine of a dog dying from bronchopneumonia was obtained in the usual way after washing and was put aside for putrefaction in the thermostat at 38° C. Putrefaction continued for four days with the production of a foul smelling material which was then heated to 60° C. for one hour, centrifuged, and filtered. The filtrate was a clear, slightly greenish, opalescent fluid with a disagreeable odor. The total filtrate (65 c.c.) was injected in divided doses. The blood pressure at the beginning was 160 mm. of mercury; the first fraction of 10 c.c. caused a drop to 50 mm., much like that following peptone injection. There was a slow return to 100 mm. during the

next ten minutes. Subsequent injections had much less effect, causing a general upward tendency of the blood pressure. There was, however, always a slight drop after small injections. At the end of seventy minutes the blood pressure rose to 130 mm. of mercury. This reaction is much like that produced by peptone injection. 12.15 P. M. Dog was removed from the kymograph. Blood taken from the jugular vein showed delayed clotting, one hour being necessary for complete coagulation.

March 28. Dog is in excellent condition.

The five preceding experiments show that the normal intestinal mucosa obtained from any part of the small intestine and allowed to undergo autolysis is without toxic effect when given intravenously to a normal dog. The filtrate prepared from the entire mucosa of the small intestine, after autolysis, may be introduced into the circulation without any signs of intoxication. In some of the experiments the autolytic fluid was not even heated to 60° C. before being centrifuged, yet the reaction was not modified. It is well known that a solution containing considerable numbers of bacteria can be given intravenously in normal dogs without ill effect. Hence this series of experiments affords a complete control to the preceding series in which the presence of a toxic substance in the mucosa of the closed duodenal loops was demonstrated.

The mucosa of the intestine after aerobic putrefaction at 38° C. for four days is not toxic. We have performed some experiments using the mucosa of the small intestine which had been permitted to putrefy under anaerobic conditions for several days. In some of these an indication of slight toxicity was obtained, but the effects are different from those given by the toxic substance present in the duodenal loop.

INTESTINAL MUCOSA FROM A DOG POISONED WITH DUODENAL LOOP FLUID IS NON-TOXIC.

Dog S–44.—Black mongrel, male; weight, 15 pounds.

April 27. Ether anesthesia and kymograph as usual. The entire intestinal mucosa obtained from dog S–40 (fatally poisoned with duodenal fluid; death in three hours) was washed and scraped off. This mucosa presented the usual swollen, velvety, purple appearance. It was completely removed and allowed to undergo autolysis for seven days at 38° C. under chloroform and toluol. It was then centrifuged and filtered and injected intravenously as usual. The total amount (135 c.c.) was given to the normal animal (dog S–44). The blood pressure at the beginning of the observation was 150 mm. of mercury. The injection of the fluid in fractions caused transient drops in blood pressure to 100 mm. with rapid return to normal. At the end of thirty-five minutes the blood pressure

was 160 mm. The pulse was regular. Dog was removed from the kymograph; there was no indication of poisoning.

May 2. Dog is well.

The preceding experiment does not indicate that the poison, when given intravenously, is excreted by the intestinal mucosa or is fixed demonstrably in this part of the intestine. The experiment serves, too, as still another control to show that the intestinal mucosa contains no toxic substance.

DUODENAL FLUID DIGESTED WITH NORMAL MUCOSA. DEATH IN FOUR HOURS.

Dog S-84.—Mongrel terrier, female; weight, 12 pounds.

July 14, 12 M. Ether anesthesia and kymograph as usual. The duodenal fluid was obtained from dog S-69 (closed duodenal loop dog; immunized; death in two and one half days). 100 c.c. of this duodenal loop fluid were added to the mucosa which was washed and scraped from the entire ileum of a normal dog. The mixture was incubated with chloroform and toluol at 38° C. for nine days and then heated at 60° C. for one hour, centrifuged, and filtered. The total filtrate was 135 c.c., but only 75 c.c. were given intravenously, as usual, in this experiment. The blood pressure curve was characteristic. At the beginning of the observation it was 125 mm. of mercury. The first injection caused a rapid drop to 60 mm., followed by a rapid return to above normal, to 160 mm., with a slow return to normal. A second fraction produced a similar result and was followed by a slow secondary fall during the next twenty minutes, reaching a level of 70 mm. at the end of forty-five minutes, at which time the animal was removed from the kymograph. 4.30 P. M. Dog found dead but quite warm. There has been much bile-stained vomitus, and an escape of yellow fluid stools.

Autopsy.—Performed at once. The heart is dilated and shows a few soft clots. The lungs and thorax are normal. The spleen is greatly engorged, with indefinite purplish areas along its margin suggesting hemorrhage beneath the capsule. The liver is deep purple and tense. The kidneys show marked congestion of the pyramids. The splanchnic vessels are conspicuous. The stomach shows some subserous ecchymoses. Its mucosa in the cardiac portion is deep reddish pink. The pylorus is pale. It contains bile-stained, watery fluid. The duodenum shows a mottled reddish congested mucosa, especially in the descending arm. The jejunum shows slight congestion and much thin fluid but no blood. The ileum contains much watery fluid, but its mucosa is normal. The large intestine shows a pinkish mucosa.

The preceding experiment shows that the duodenal loop fluid contains a toxic substance which is not neutralized or destroyed by autolysis with the mucosa of the normal ileum. It does not, however, prove that there can be no neutralization *in vivo,* as claimed by Draper-Maury, but speaks against the possession of very powerful neutralizing action by the mucosa. We shall return to this point later.

CLOSED DUODENAL LOOP WITH MERCURIC CHLORIDE AND TOLUOL.

Dog S–55.—Strong mongrel, male; weight, 21 pounds.

May 21, 3 P. M. Ether anesthesia and operation as usual. Closed duodenal loop isolated, washed with water, and then rinsed out thoroughly with a (1:10,000) solution of bichloride of mercury. After the last washing, 5 c.c. of toluol were introduced and left in the loop.

May 22, 11 A. M. Dog looks well and there is no vomiting. Rectal temperature, 38.5° C.

May 23, 12 M. Dog is lying on its side and appears badly shocked. The pulse is weak. Temperature, 37.2° C. 3 P. M. Rectal temperature, 38° C. Ether anesthesia and kymographic record from the carotid. The blood pressure is 50 to 55 mm. of mercury, with a slight tendency to further fall, reaching 45 mm. at times. Intravenous injection of adrenalin gave the characteristic rise of blood pressure to 200 mm., and slowing of the heart beat. At the end of twenty-five minutes the blood pressure measures 55 to 60 mm. At this time the intestine was opened and inspected. Throughout the upper part the mucosa of the small intestine presented a mottled reddish appearance. The animal was killed by bleeding from the carotid.

Autopsy.—Performed at once. The heart, lungs, and thorax are normal. The spleen and liver are greatly congested. The stomach is full of bile-stained fluid, and the cardiac portion is a little congested. The duodenum between the pylorus and the upper end of the loop shows some reddening of the mucosa. The jejunum and ileum show the same mottled reddish mucosa as before death, with occasional ecchymoses. The closed duodenal loop shows some hemorrhage below the serous coat and fine grains of fibrin on the surface. The mucosa is of a diffuse pinkish color with shallow ulcers forming in the mucosa opposite the mesenteric attachment. The loop contains about 75 c.c. of slimy, dark greenish material, containing flakes of mucus. There is no objectionable odor. Cultures gave moderate growths of bacilli and cocci. Smears show many pus and epithelial cells, numerous bacteria of various sizes, and numbers of cocci.

The preceding experiment and others of similar nature were performed in an attempt to remove the bacterial element or at least to check the growth of bacteria in the closed loop. It will be seen that this attempt failed and the dogs developed the usual toxic symptoms with low temperature and blood pressure and presented the usual findings in the intestinal tract. The loops at autopsy contained numerous bacteria.

MUCOSA DESTROYED IN A CLOSED DUODENAL LOOP. TOXIC SUBSTANCE NOT PRESENT.

Dog S–65.—Young mongrel, female; weight, 14½ pounds.

June 16, 3 P. M. Ether anesthesia and operation with isolation of a closed duodenal loop. The closed loop was washed out carefully with water and then with a 4 per cent. solution of sodium fluoride. This solution of fluoride was allowed to remain in contact with the mucosa for ten minutes. Then a second injection was made lasting fifteen minutes, the solution being in contact with the

mucosa for twenty-five minutes in all. Then the closed loop was washed out thoroughly with water and the puncture wounds were closed as usual.

June 17, 11 A. M. Dog is pretty well, but refuses food. 3 P. M. Dog is dead.

Autopsy.—Performed at once. The heart and thorax are negative. The lungs are voluminous and moist, and show indefinite patches of bronchopneumonia. The spleen is a little swollen. The liver is a trifle congested. The kidneys are negative. The stomach is distended with bile-stained fluid and shows some congestion. The small intestine shows considerable fluid, and its mucosa shows congestion. The duodenal loop is distended with about 100 c.c. of bloody fluid, having a disagreeable odor. The loop has purplish areas showing through the serous surface. The mucosa is found to be greatly degenerated and scrapes off with great ease showing submucous hemorrhages. The duodenal fluid together with the mucosa was preserved with chloroform and toluol, and tested in dog S–76.

Microscopical Examination.—The liver is negative. The duodenal loop mucosa shows practically complete necrosis of the epithelial covering, only a few epithelial cells remaining in the bottom of a few crypts. The mucosa is replaced by a thick layer of polymorphonuclear leucocytes and cellular debris. The submucosa shows extensive hemorrhage, and the subserous tissue shows an acute exudate of polymorphonuclear leucocytes and fibrin with a recent fibrinous peritonitis, little focal abscesses developing here and there. The jejunum shows a relatively normal mucosa except for slight congestion of the tips of the villi. The cause of death in this dog was evidently mucosal epithelial necrosis, bacterial invasion of the peritoneum and blood stream, bronchopneumonia, and edema of the lungs.

Dog S–76.—Yellow puppy, female; weight, 9 pounds.

July 8, 11.40 A. M. Ether anesthesia and kymograph as usual. Intravenous injection of duodenal loop fluid from dog S–65, in which the duodenal mucosa had been killed with sodium fluoride. The material obtained from the closed loop had undergone autolysis for three weeks at 38° C., and had been heated at 60° C. for one hour and centrifuged. The clear, supernatant, slightly blood-stained fluid was injected intravenously. The total filtrate amounted to 65 c.c. and caused relatively little change in blood pressure. The pressure before injection was 140 mm. of mercury. There was a slight initial fall followed by an extreme rise above normal to 220 mm. This fell rapidly to normal, and the same reaction took place after the second fraction was introduced. At the end of twenty minutes the blood pressure measured 120 mm. 12 M. Dog was removed from the kymograph. 2 P. M. Dog is pretty well. 10 P. M. Dog is normal.

July 9. Dog is normal and gave not the slightest indication of intoxication following this injection.

The two preceding experiments show that by suitable chemical injury (sodium fluoride) the mucosa of the closed loop can be almost completely destroyed. Under these conditions the dog dies rapidly because of bacterial growth and invasion of the peritoneum and blood stream, and a terminal bronchopneumonia and edema of

the lungs. The duodenal loop fluid which accumulates under these conditions may be looked upon as a simple inflammatory exudate, and is shown to be free from the toxic substance present in the usual closed duodenal loop. This duodenal loop fluid is different in many respects from the usual loop fluid, but as seen above, it can be given intravenously without any toxic effects. This is the final proof that the toxic substance is elaborated by the activity of the mucosa and cannot be produced by bacterial growth and inflammatory reaction alone.

CLOSED DUODENAL LOOP WITH DESTRUCTION OF THE MUCOSA. TOXIC SUBSTANCE NOT PRESENT.

Dog S–81.—Mongrel terrier; weight, 14 pounds.

July 9, 4 P. M. Ether anesthesia and isolation of the closed duodenal loop in the usual way. The loop was washed out with water, then 35 c.c. of 4 per cent. sodium fluoride solution were introduced and retained for ten minutes. This was washed out with water and a second fraction of sodium fluoride was introduced and retained for ten minutes. This was washed out thoroughly with water and the loop was closed as usual.

July 10, 10 A. M. Dog is vomiting repeatedly. 12.30 P. M. Dog is lying on its side. The pulse is weak. 1 P. M. Blood culture, made from the jugular vein, is sterile. 3 P. M. Dog is weak and toxic. 4.30 P. M. Animal died.

Autopsy.—Performed at once. The thorax is opened and blood is obtained under aseptic precautions from the right ventricle. Cultures are again sterile.

The thorax is negative. The heart is contracted. The lungs are voluminous. and the right lung is specked with small hemorrhagic areas 2 or 3 mm. in diameter. The spleen, kidneys, pancreas, and liver are negative. The peritoneum contains about 10 c.c. of bloody fluid, and shows a beginning peritonitis with ecchymoses on the serous surface. The loop is greatly distended and shows a mottled red and purple coloring through the serosa. It contains about 100 c.c. of brick red fluid in which float flakes of mucus. The mucosa seems intact, but is easily scraped off, and extensive hemorrhage occurs in the submucosa. The material replacing the loop mucosa is almost as soft as moist blotting paper. The fluid of the loop together with the scraped off mucosa were autolyzed with toluol and chloroform (dog S–88).

Microscopical Examination.—The duodenal loop shows necrosis involving the greater part of the mucosa; perhaps one fifth of the epithelial covering still retains its original form. The outer zone, however, is a solid mass of leucocytes, red blood cells, bacteria, and cell debris. The intestinal coats show hemorrhage, and the peritoneal surface shows beginning peritonitis with an exudate of fibrin and pus cells. The kidneys and liver are normal. The lungs show edema and patches of hemorrhage with exudation of leucocytes, but no fibrin. Numbers of bacteria can be seen in the sections of lung tissue.

Dog S–88.—Fox terrier, female; weight, 11 pounds.

July 12, 5.15 P. M. Ether anesthesia and kymographic observation. Intra-

loop whose blood supply has been in no way disturbed, and whose mucosa is quite intact both in gross and microscopically. They state that the toxic substance is purely bacterial in origin and probably not dependent on any vital secretion of the mucous membrane. This statement we believe is not proven by their experiments, and is controverted by ours, which show clearly that the mucosa is absolutely essential for the production of the toxic product.

The toxic substance can be demonstrated in the mucosa of the closed loops after a lapse of twenty-four hours following the operation. It is very difficult to give a clear explanation of this process of its formation, but we may assume with reason that the enormous growth of intestinal bacteria is a very important stimulus to the perverted activity of the intestinal mucosa. It is possible, too, that the absence of secretions from the stomach is an important factor in the reaction process. Until we have a better understanding of this phenomenon it may be permissible to speak of the mucosa being upset or developing a perverted activity with formation of a toxic substance which may be absorbed directly into the blood. The dogs with drained duodenal loops may live for months under suitable conditions and we must assume in this instance either a strong immunity or a recovery of the mucosa with return to a relatively normal activity. The histories of some of the dogs with drained duodenal loops have been given in the preceding paper and will not be reviewed here.

The point that comes up in connection with the intoxication following the production of a drained loop is of considerable importance in connection with cases of human intestinal obstruction. Human cases of obstruction in which an enterostomy has been performed some distance above the obstruction may be looked upon as having a drained intestinal loop made up of the bowel between the obstruction and the enterostomy wound. In such cases there is no circulation of gastric, pancreatic, and hepatic secretion in the isolated loop, and in spite of drainage we may assume a possible upset of the mucosa with production of a toxic substance and consequent intoxication. The clinical diagnosis in such cases will point to a second intestinal obstruction; but autopsy may show that such is not the case. We expect to furnish evidence to support this view

in a forthcoming report dealing with cases of obstruction in human beings.

SUMMARY.

The blood of closed duodenal loop dogs is not toxic to normal dogs. The blood of dogs that have been fatally poisoned with duodenal loop fluid is likewise non-toxic to normal dogs.

The mucosa of closed or drained duodenal loops contains a toxic substance quite similar to the toxic material found in the lumen of the closed loops.

This toxic substance is absorbed from the mucosa itself and not from the lumen of the drained loops. The same is probably true of the closed loops which have an intact mucosa. It seems highly probable that the poison is formed by the mucosa and is in great part absorbed directly from it by the blood.

Normal intestinal mucosa contains no toxic substance nor can it neutralize *in vitro* the toxic substance produced in the closed loops.

There is no evidence that the toxic material when given intravenously is excreted by the intestine or held by the intestinal mucosa in any demonstrable form.

The toxic substance is not absorbed from the normal intestinal tract.

Destruction of the mucosa in a closed loop by means of sodium fluoride prevents the formation of the toxic substance. This fact furnishes the final proof that the mucosa is the essential factor in the elaboration of the poisonous material.

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THE EXTRACELLULAR RELATION OF THE MALARIAL PARASITE TO THE RED CORPUSCLE, AND ITS METHOD OF SECURING ATTACHMENT TO THE EXTERNAL SURFACE OF THE RED CORPUSCLE.*

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PLATES 56 TO 61.

Since the malarial parasite was first described in 1880, many views have been held as to the morphology of the ring-form parasite, and its possible formation and significance, but with a remarkable similarity of opinion among observers as to the parasite being intracellular.

Laveran (1) has consistently claimed from the beginning that the parasites were extracellular and that they attached themselves to the surface of the red corpuscles, adhering to them "by pressing on them."

Richard (2) thought at one time that the parasites were within the substance of the red corpuscle; but later (3) he returned to Laveran's opinion that they were attached to the surface.

Mannaberg (4) inclines to the opinion that the young unpigmented parasites "lie for a considerable time upon the blood corpuscles. . . . In the places where the parasites were observed, distinct impressions in the substance of the blood corpuscles are noticed, and that the margin of the so-formed depression is perfectly sharp. . . . If these bodies were within the substance of the blood corpuscle, one cannot understand how the observed depression in the upper surface or edge of the blood corpuscle takes place."

Marchiafava and Bignami (5) describe a similar condition, depicting the parasites as being embedded or pressed into the surface of the red corpuscle; but these observers believe the greater number of parasites, even the young forms, to be endoglobular.

When Koch was in this country he was shown models of the red corpuscles with the malarial parasites beneath the surface, and he made the statement that the parasites were not within the substance of the red corpuscle but attached to the surface.

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THE METHOD BY WHICH THE MALARIAL PARASITE SECURES ATTACHMENT TO THE OUTSIDE OF THE RED CORPUSCLE.

The malarial parasite is extracellular during its entire developmental cycle; that is, with the exception of the brief periods when it is free in the blood serum, it is attached to the outside surface of the red corpuscle.[1]

The parasite secures its attachment to the outside of the red corpuscle by means of filamentous pseudopodia thrown out by the parasite for that purpose. The attaching processes will be described as (1) primary and (2) secondary (accessory) attaching pseudopodia.

(1) *Primary Attaching Pseudopodia.*—(Plates 56 to 61). These are delicate thread-like processes, arising from the cytoplasm of the parasite, near its nucleus (plate 56, figure 2; plate 59, figure 1). The parasite appears to secure its primary hold on the red corpuscle by means of these pseudopodia; but as the corpuscular mound to which the parasite is attached becomes dehemoglobinized, or decolorized, or as the parasite increases in size, accessory, or secondary attaching pseudopodia are formed.

(2) *Secondary Attaching Pseudopodia.*—These may be thrown out from various portions of the cytoplasm of the parasite, apparently for the purpose of securing a firmer hold on the red corpuscle (plate 58, figure 7, x; plate 59, figure 15, x).[2] The pseudopodia can be seen overlying the red corpuscle in the form of a loop, while the body of the parasite, with its nucleus, may lie either on the surface of the red corpuscle (plate 58, figures 1 and 2, and 4 to 10) or along its periphery in close apposition to the corpuscle (plate 56, figures 6 to 11). Occasionally, especially in the very young parasite, the loop may be formed from the entire protoplasm of the parasite (plate 59, figure 9).

The parasite squeezes up that portion of hemoglobin substance which lies within the boundary of this loop into a mound, circular at the base, with a more or less rounded apex (plates 56 to 59). The

[1] The red corpuscle will be described in this article as having an upper and an under surface, and a periphery.

[2] The accessory filaments are especially common to the tertian parasite, even in the earliest stages of its development.

parasite when thus attached maintains its position on the outside of the red corpuscle by means of these pseudopodia encircling the mound of corpuscular substance at its base.

When viewed from above the young parasites with their pseudopodia encircling mounds of corpuscular substance give the characteristic ring-form pictures (plate 56, figures 7 to 10; plate 58, figures 1 and 2, 4 to 10, and 12 and 13; plate 59, figures 9 and 10; plate 60, figures 1 to 4), which have been so variously interpreted. This interpretation of the ring-form parasite explains the absence of pigment in the corpuscular mound area and makes it clear why it has been found impossible to stain this area.

The true interpretation of these ring-forms might be difficult if the hemoglobin mounds were not seen projecting outward from the periphery of the red corpuscle, surrounded by the parasites (plate 56, figures 13 to 35; plate 57 ,figures 1 to 23, 27 and 35; plate 58, figures 3, 11, 14, and 26 to 29; plate 59, figures 2, 4, and 11 to 21; plate 61, figures 5 to 9, and 11). But the fact that they are seen in this position makes the interpretation easy, especially as the delicate, thread-like pseudopodia can be seen encircling the hemoglobin mound. Very often the encircling pseudopodium can be traced in its entirety around these peripheral mounds (plate 56, figures 18 to 20, and 29; plate 57, figures 16, 32, and 35; plate 58, figures 26 and 27).

Occasionally a part of the encircling filament is obscured by the thickened mound of corpuscular substance overlying it, this being due to the fact that the mound has been flattened or pressed down in the process of smearing the blood (plate 57, figures 26 to 28, and 30 and 33; plate 58, figures 4 to 6); but careful focussing generally allows even these portions of the pseudopodium to be seen. When studying the parasites in fresh blood preparations, portions of parasites lying beneath these pressed down mounds of corpuscular substance might lead one to think that the parasites were within the substance of the red corpuscle.

With very few exceptions, the adult parasite is attached to the surface rather than to the periphery (plate 60, figures 13 and 14) of the red corpuscle. I believe that the explanation of this is that the surface of the red corpuscle affords a larger area for occupation

as well as for absorption. In this situation, moreover, the chances for the forcible removal of the parasite from the red corpuscle are fewer.

When the parasite is attached to the under surface of the red corpuscle (plate 58, figure 14, A) the blue staining protoplasm of the parasite viewed through the red corpuscle is paler and the outlines of the parasite are less distinct than when the parasite is seen on the upper surface of the red corpuscle (plate 58, figure 14, O). When two parasites are attached to the one corpuscle, one on either surface, these differences can be well seen (plate 58, figures 14 and 26). Also the same differences can be noted when a single parasite is seen on both surfaces of the red corpuscle (plate 57, figures 7, 14, and 22; plate 59, figures 4 and 5).

When a parasite, especially an adult, is attached to the under surface of a red corpuscle, the nucleus may be indistinct or obscured from view by the overlying, blue-stained protoplasm of the parasite, and may give the impression of a parasite without a nucleus. And the nucleus of a free parasite may be hidden from view, if it is on the under surface of the parasite.

Occasionally an adult parasite is attached to a corpuscular mound in a position so close to the periphery of the red corpuscle that the nucleus of the parasite projects beyond the periphery (see plate 60, figure 15, where the nucleus of the parasite extends beyond the periphery of the corpuscle to which the parasite is attached, and overlies the red corpuscle adjoining the infected one). It is not at all uncommon to see the nucleus of the young parasite of the æstivo-autumnal infections projecting beyond the periphery of the red corpuscle to which the parasite is attached (plate 56, figures 13 and 32; plate 57, figures 12 and 15; plate 58, figures 6 and 7), and occasionally the nucleus of a tertian ring may be seen beyond the periphery of the red corpuscle (plate 59, figure 7). The blue-stained protoplasm of a parasite may also be seen beyond the periphery of the corpuscle to which the parasite is attached (plate 56, figures 6 to 12, and 14 to 17, and 22; plate 58, figure 25; plate 59, figure 3).

Ewing (6) has noted the fact that portions of a parasite may go beyond the periphery of the corpuscle to which the parasite is attached: "In many tertian

cases, the body and especially the nucleus of the parasite appear to project beyond the border of the cell, even more distinctly than in the case of the æstivo-autumnal ring."

Often when a parasite has attached itself to a corpuscular mound, that portion of the nucleus of the parasite which is next to the mound may be seen to have conformed itself to the circular shape of the mound (plate 57, figures 34 and 35).

Retraction of Hemoglobin.—This is a condition caused, I believe, by the parasite which, in the process of pulling up the corpuscular mound for the purpose of attaching itself to the red corpuscle, draws up a portion of the hemoglobin from the rest of the corpuscle in such a manner as to leave decolorized areas at the periphery of the corpuscle, the contour of the corpuscle remaining undisturbed (plate 57, figures 11, A, and 12, A).

In discussing this retraction of the hemoglobin, Thayer and Hewetson (7) state: "In some cases we may see corpuscles containing small, refractive, *ring-like* bodies, with or without pigment, where the hemoglobin seems to have left the outer part decolorized though still showing the outline of the original corpuscle." Craig (8) has also noted these colorless areas, describing the condition as "retraction of hemoglobin."

The Formation of Rings by the Joining of Pseudopodia.—This has been noted by several observers.

Thayer and Hewetson (9) have observed that a small parasite may form a ring through the meeting and fusing of pseudopodia, and noted that in the middle of this ring there lies, apparently, a bit of corpuscular substance. Thayer (10) states that "some observers believe that such a portion of the corpuscle included within two pseudopodia is gradually digested by the parasite."

DaCosta (11) writes that rarely the ring may be formed by the fusion of two short pseudopodia between which a small portion of the corpuscle may become imprisoned, and Ewing (12) states that "secondary" (accessory) rings sometimes form from the union of pseudopodia, and that these are identical in appearance with the primary ring, but lack the chromatin granules." Osler, Councilman, and Antolisei (13) believed that some of the ring-forms were small parasites with portions of the red corpuscles included within their substance. Marchiafava and Bignami (14) do not deny that there are parasites which have included a portion of the red corpuscle, but they believe it to be an atypical process.

In view of these facts Mannaberg's (15) observations are interesting: "If such a ring is observed with an open Abbe and oblique illumination with a concave mirror, it is definitely seen to produce in the upper surface of the blood

corpuscle a deep and sharp depression, and in the center the substance of the blood corpuscle projects through the hoop just as a finger through a ring."

THE DECOLORIZED CORPUSCULAR MOUND, OFTEN CALLED THE ACHROMATIC AREA OR MILKY ZONE.

The terms achromatic area, or milky zone, have been applied to the decolorized, transparent, and pigment-free corpuscular area encircled by the filamentous pseudopodium of the parasite.

The decolorization of the corpuscular mound is gradual, proceeding as the parasite digests the hemoglobin. All stages between the deeply colored mound of unaltered hemoglobin and the completely decolorized one have been observed and can be followed with the microscope in fresh blood as well as in stained specimens (stages between A and O, plate 58, figure 7).

Complete decolorization, or dehemoglobinization, of corpuscular mounds surrounded by parasites may be seen in red corpuscles showing Schuffner's granulation (plate 60, figure 1), and in red corpuscles with blue stippling (plate 60, figure 4), suggesting that these granulations form a part of the corpuscular substance digested by the parasite.

The decolorization of the corpuscular mound by the young ring-form parasite, especially of the æstivo-autumnal infections, may be complete before the appearance of any demonstrable pigment in the protoplasm of the parasite.

It has been believed that the achromatic area was an essential part of the malarial parasite, and it has been variously described as a vesicular nucleus, a digestive vacuole, and a thinning of the protoplasm of the parasite which allowed the red corpuscle to show through. When certain of these decolorized areas were seen at the periphery of the red corpuscle (plate 57, figures 24 and 25; plate 58, figures 12 and 13), they were pictured and described by Mannaberg and Marchiafava and Bignami, who interpreted them as extracellular parasites resting in niches at the periphery of the red corpuscle.

That the colorless, or dehemoglobinized, corpuscular area surrounded by the parasite is more or less transparent seems proven by the following facts: (*a*) When the achromatic area of one corpuscle

partially overlies the periphery of another red corpuscle, this periphery can be distinctly seen through the achromatic area (plate 58, figure 12). (*b*) When the achromatic area of one red corpuscle partially overlies that of another red corpuscle, the nucleus and the protoplasm of the parasite surrounding the underneath decolorized area is clearly seen (plate 58, figure 13). (*c*) When the smear is made on the cover-slip, precipitation of stain may be seen more or less clearly through the decolorized corpuscular mound. (*d*) When the parasite-infected corpuscles are piled up, one completely over another, it is impossible to find any completely decolorized corpuscular mounds, although many decolorized areas surrounded by parasites may be seen where the smear is thinly spread.

The fact that the area which is surrounded by the parasite is sometimes seen to be achromatic and transparent, when other portions of the infected corpuscle show good color, proves that this area is not parasitic. For if this area belonged to a parasite which was attached to a deeply colored red corpuscle (plate 58, figures 12 and 13), the area would have to be opaque in order to be achromatic, otherwise the color of the corpuscle would show through it more or less clearly.

The gradual decolorization of the corpuscular mound undoubtedly has given rise to many conceptions regarding the finer structure of the ring-form parasite.

For example, many observers believe the rings to be ameboid bodies, which, becoming very thin at the center or being palely stained or unstained, permit the corpuscle to show through, thus giving the parasite the appearance of being ring-shaped. I believe this view is held by Marchiafava and Celli (16), Celli and Guarnieri (17), Mannaberg (18), Thayer and Hewetson (19), Bastienelli and Bignami (20), Ziemann (21), and Opie (22), and it is the view formerly held by the author (23).

Argutinsky (24) interpreted the ring-form parasites as artefacts, and Nocht (25) believed them to be optical illusions due to discoid bodies with thickened rims.

Others held the view that the achromatic area was a bladder-like nucleus in which lies a small, deeply staining chromatin mass. Among those who incline to this view, are Sakharoff (26), Canalis (27), Celli and Sanfelice (28), Grassi and Feletti (29), Romanovsky (30), Manson (31), Osler (32), Cabot (33), and Craig (34).

Still other observers, Marchiafava and Bignami (35), Schaudinn (36), Maurer (37), Calkins (38), Loeffler (39), and others, believe the achromatic area, or decolorized corpuscular area, to be a nutrition vacuole.

In depicting the changes in color of the corpuscular mound, Marchiafava and Bignami (40) state: "The ring surrounds a space which takes the same stain as the red corpuscle, especially in its youngest stage, while in the center of more developed forms the red corpuscle is of paler appearance than it is outside of the ring" (digestion of the hemoglobin by the parasite). "There is, therefore, in this form a very diaphanous portion of the parasitic body which prevents a perfect appreciation of the color of the corpuscle. . . . The blue ring is the protoplasm of the parasite which includes a nutritive vacuole."

After a parasite has completely decolorized, or dehemoglobinized, a red corpuscle, it usually assumes a compact form,—the ring shape is lost and either a very small attaching area is seen or the pseudopodia are entirely withdrawn by the parasite. I believe this to be a stage preparatory to migration. Bass and Johns[3] possibly refer to a similar appearance: "When parasites die . . . the central clear space in the small ring closes, or if the parasite is older, the irregular projections, pseudopodia, are withdrawn."

The Parasitic Achromatic Area.—This area closely surrounds the chromatin substance in the young ring-form parasite (plates 56 to 58), and in some instances shows quite distinctly in contrast with the deeply colored corpuscular substance (plate 57, figure 4; plate 59, figure 3). The parasitic achromatic area increases in size with the growth of the parasite (plate 60, figures 5 to 12, and 14 and 15). This area is not always achromatic (plate 60, figure 11), but may stain either a delicate blue (plate 60, figure 15), or a pink (plate 60, figures 9 and 10).

When this area surrounding the chromatin substance of the parasite is unstained, and overlies the area of corpuscular substance which has been decolorized by the action of the parasite surrounding it, it may be impossible to differentiate the one colorless area from the other; the chromatin mass may thus appear to be separated from the blue-stained protoplasm of the parasite and to lie within and belong to the colorless corpuscular area, thus giving rise, probably, to the erroneous belief that the colorless corpuscular area is a part of the parasite itself (plate 59, figures 25 and 26). But even in these instances a careful scrutiny of the parasite will often show a slight differentiation between the colorless corpuscular area and

[3] Bass, C. C., and Johns, F. M., *Jour. Exper. Med.*, 1912, xvi, 567.

the achromatic area belonging to the parasite (plate 58, figures 8, 12, and 13).

The latter area may stand out clear and sharp against the unstained background of the blood smear when the chromatin mass of the parasite extends beyond the periphery of the red corpuscle to which the parasite is attached (plate 56, figures 12, 13, and 34; plate 58, figure 7, A), or when the parasite is free (plate 60, figures 12 and 17) in the blood serum.

That this achromatic area which closely surrounds the chromatin mass of the parasite, and which is probably a part of the nucleus, is sometimes opaque or not entirely transparent is shown by the fact that this area can be seen when the infected corpuscles overlie one another.

Infection of a Corpuscular Mound by Two or More Parasites.—Infection of a corpuscular mound by more than one parasite (plate 58, figures 11, and 14 to 34; plate 59, figures 7, 8, 20, 25, and 26) may be seen in all the malarial infections. The best examples are observed in the æstivo-autumnal infections. As might be expected, instances of multiple infection are most frequently met with in cases where the parasites are numerous.

The majority of young parasites occupying the one corpuscular mound are in similar stages of development (plate 58, figures 15 to 21, 23, 25, 27, and 30 to 33; plate 59, figures 7, 8, 20, 25, and 26). This similarity in size is easily explained, as the parasites are probably of one brood, and it may be the result of one segmenting parasite. Parasites of varying sizes occupying the one corpuscular mound (plate 58, figures 11 and 22) may be explained by supposing that a young parasite attaches itself to a corpuscular mound already occupied by a parasite of an older brood.

The parasites seem to follow no rule as to their position in relation to each other in the occupancy of the one corpuscular mound (plate 58). And I have found no evidence of conjugation. The bodies or nuclei do not coalesce, as with careful examination with an apochromatic lens of high power the parasites can be differentiated, one from the other, even in some of the cases where the parasites are actually superimposed (plate 58, figures 21, 29, 30, and 31).

In cases of multiple infection of a corpuscular mound, the parasites seem to proceed with the destruction of the mound in the same way that the single parasites do. Occasionally one of the young parasites may show a grain of pigment in its protoplasm (plate 58, figure 25).

Multiple infection of a corpuscular mound should not be confused with the appearance produced by a single parasite whose protoplasm is intact, but whose nucleus is broken up by the technique used in spreading the smears. Such parasites with nuclei forcibly separated into parts, which may be of varying sizes, are usually found along the edges of smears made on slides, and the interpretation is made easy by the fact that parasites with nuclei similarly distorted may be found in groups. A young parasite never has more than one nucleus.

The Supposed Conjugation of Parasites.—The close approximation of two parasites occupying the same corpuscular mound has undoubtedly led to many conceptions or theories as to the conjugation of malarial parasites; but the fact that the corpuscular mounds occupied by more than one parasite may be seen at the periphery of the infected red corpuscle (plate 58, figures 11, 14, and 26 to 29) makes my interpretation less difficult than it might otherwise be.

Mannaberg (41), in 1894, described what he believed to be a process of conjugation; in the first he shows the young ring-form parasites coalesce with each other to form larger bodies, these latter later developing into crescents. He pictured, as an early stage in the process, two young ring-form parasites side by side, usually at the periphery of the red corpuscle. "The outer contour of both parasites frequently coalesces without interruption, and falls together with the edge of the blood corpuscle; the inner contour is often of a concave shape." This description, as well as Mannaberg's illustration of the same, corresponds with that of two young ring-form parasites attached to adjoining corpuscular mounds on the one red corpuscle at its periphery (plate 58, figure 8). Two young ring-forms, attached to the same corpuscular mound, with the bodies of the parasites at opposite poles, may resemble a small crescent (plate 58, figure 33).

Ewing, in 1901, described a process of conjugation between the parasites of tertian infections. He was led to believe that the parasites conjugated, because in certain smears of malarial blood studied by him nearly all the younger parasites were twinned, while all the older forms were single. These facts are explained by the migration of the younger malarial parasites to other corpuscles. He describes the process of conjugation as beginning with two young parasites

of varying morphology, depicting first a union of the protoplasm of the young parasites, the fusion of the nuclei taking place at a later stage in the development of the conjugating pair. In illustration of the first stage he pictures two young parasites, side by side, each attached to its own corpuscular mound, with their peripheries touching or overlying. A later stage illustrates adult parasites with two or three groups of chromatin granules "surrounded by one achromatic zone."

This last stage represents, I believe, the fastening of two or three adult parasites, by the pseudopodia arising near their nuclei, to the one corpuscular mound, which has become decolorized by the action of the parasites (plate 59, figures 25 and 26). The groups of chromatin granules are separated from each other and from the parasitic body by the achromatic areas belonging to the parasites.

The appearance of fusion between the protoplasm of two adult parasites is seen where two accessory pseudopodia encircle the same corpuscular mound, one being superimposed over the other.

In 1905 Craig (43) described a process of "intracorpuscular conjugation" of young ring-form malarial parasites. He figures three stages in the conjugation process: in the first, he shows the young ring-form parasites, side by side, their protoplasm touching or overlapping at the periphery; in the second stage, besides the parasites described in the first stage, he pictures what appears to be two young parasites occupying the same corpuscular mound; and in the third stage he shows (*a*) parasites attached to the corpuscle on separate mounds but in close proximity, (*b*) what appear to be parasites with distorted nuclei, (*c*) multiple infection of corpuscular mounds, and (*d*) large ring-form parasites with single nuclei. He interprets the last as resulting from conjugation.

Craig's description of the morphology of these parasites corresponds with that of the two parasites occupying one corpuscular mound, with the exception, perhaps, that he has never observed the presence of pigment in the protoplasm of these parasites. In discussing his disagreement with Ewing's theory, he states that he does not see any difference in the morphology of the conjugating parasites, for "while, in rare instances, one of the organisms may appear *slightly larger than the other,* may have a larger mass of chromatin, and *stain more intensely,* in the vast majority of instances the two conjugating parasites are of exactly the same size, and cannot be distinguished one from the other in any way."

I have not been able to find any evidence of conjugation of malarial parasites in any stage of development, and it may be that Craig's "conjugating parasites" are the result of a wrong interpretation of the infection of a corpuscular mound by two young parasites. The idea that the parasites were intracellular perhaps led to the misconception.

FREE PARASITES WITH ATTACHING PSEUDOPODIA IN THE FORM OF LOOPS.

The majority of free parasites seen in malarial infections have a compact structure. Rarely free parasites are seen with pseudopodia extended for attachment (plate 56, figures 2 to 5; plate 59, figures 1 and 6), and occasionally free parasites are seen with their pseudopodia in the form of loops, with or without the nuclei at the extremity of the loops (plate 60, figures 16 to 22). These free parasites with the loop-form pseudopodia are in migration, and have been set free while their pseudopodia are still in the form assumed by the parasites for attachment to corpuscular mounds (plate 60, figure 23).

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EXPLANATION OF PLATES.

THE EXTRACELLULAR RELATION OF THE MALARIAL PARASITE TO THE RED CORPUSCLE. THE METHOD BY WHICH THE PARASITE SECURES ITS ATTACHMENT TO THE SURFACE OF THE RED CORPUSCLE.

PLATE 56.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,900.

FIG. 1. The youngest form of the æstivo-autumnal parasite, resulting from a recent segmentation. This figure is used as a standard of comparison as to the relative sizes of the parasites illustrated.

FIGS. 2 to 4. Young parasites free in the blood serum, showing filamentous pseudopodia arising from the cytoplasm of the parasites. Fig. 2 shows well the origin of the pseudopodia near the nucleus of the parasite.

FIG. 5. A young parasite, free in the blood serum, showing the attaching pseudopodium in the form of a loop.

FIGS. 6 to 11. Early stages in the attachment of the parasite to the red corpuscle by means of the pseudopodium arranged in a loop, overlying the corpuscular substance. The parasitic body is on the periphery of the red corpuscle.

FIG. 12. A young parasite on the periphery of the red corpuscle, starting to pull up the corpuscular mound.

FIGS. 13 to 35. Parasites in various positions attached to the surface of the red corpuscle by means of their delicate, thread-like pseudopodia encircling mounds of corpuscular substance at the periphery of the red corpuscle. The mound of corpuscular substance has been pulled up by the parasite apparently for the purpose of attachment and digestion. Note that the surrounding filament can be seen in its entirety in many of the figures (i. e., Figs. 18, 19, 20, and 29). In many of the parasites an achromatic area can be seen closely surrounding the chromatin granule of the parasite. Figs. 26, 33, and 35 show minute granules of pigment in the cytoplasm of the parasites. Fig. 22 shows the parasite overlying the corpuscular mound. The encircling filament can be seen at o.

PLATE 57.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,900.

FIGS. 1 to 10. Parasites attached to the external surface of the red corpuscle by means of their pseudopodia which encircle the corpuscular mounds at the periphery of the red corpuscles, the mounds having been pulled up by the parasites for the purpose of attachment. The pseudopodium of the upper parasite in Fig. 2 can be seen encircling the peripheral mound. In Fig. 7 the parasite can be seen on both surfaces of the corpuscle. The part of the parasite on the under surface can be seen at o.

FIGS. 11 and 12. These figures show, I believe, what has been described as "retraction of hemoglobin"; *i. e.*, clear unstained areas at the periphery of the corpuscles. This condition is due to the fact that the parasite, in attaching itself to the corpuscle, has pulled up the hemoglobin in such a manner as to leave decolorized areas at the periphery of the cell. The condition is not the result of destruction of the hemoglobin substance. At o in Fig. 12, two parasites can be seen surrounding the mound of hemoglobin which has been pulled up from the clear areas at A.

FIG. 13. A parasite attached to a peripheral corpuscular mound.

FIG. 14. A portion of the body of the attached parasite on the under surface of the corpuscle at oo.

FIG. 15. The protoplasm of the attached parasite conforming to the shape of the corpuscular mound is seen.

FIG. 16. A parasite attached to a hemoglobin mound near the periphery of the corpuscle. The pseudopodium of the parasite can be seen encircling the mound at its base.

FIGS. 17 to 23. Parasites attached to peripheral corpuscular mounds which are in various stages of dehemoglobinization (pallor) due to the gradual digestion of the hemoglobin by the parasites.

FIG. 21. Crenation of the corpuscular mound. Fig. 22 shows the parasitic body on both surfaces of the corpuscle.

FIGS. 24 and 25. Parasites attached to peripheral decolorized corpuscula: mounds which have been completely dehemoglobinized by the action of the parasites. The decolorization does not extend below the encircling pseudopodia.

FIGS. 26 to 32. Parasites attached to the upper surface of the red corpuscles by means of pseudopodia surrounding corpuscular mounds. Figs. 26, and 29 to 32 show the mounds pressed down over the surface of the red corpuscles. Fig. 32 shows the pseudopodium of the parasite encircling the mound.

FIG. 33. Here the parasite appears to have pulled up the entire lower periphery of the red corpuscle into the mound to which it is attached.

FIGS. 34 and 35. Parasites attached to corpuscular mounds, one on the surface, the other at the periphery of the red corpuscle. The nuclei of these parasites conform to the contour of the mounds.

PLATE 58.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,900.

FIGS. 1 to 6. Parasites attached to corpuscular mounds. Fig. 3 shows the mounds at the periphery of the corpuscle; the other figures show the mounds on the surface of the corpuscles. When the parasites are attached to the red corpuscles in the latter position, with their pseudopodia surrounding the corpuscular mounds, the characteristic ring-form picture is seen.

FIG. 7. Two parasites attached to corpuscular mounds on the surface of the red corpuscle. The parasite at A has recently attached itself and the hemoglobin of the mound is still unaltered. At o the parasite is seen encircling a completely decolorized mound, with an accessory filament attached to another mound at x.

FIG 8. Two parasites surrounding with their pseudopodia completely decolorized corpuscular areas. This figure corresponds to a stage in the formation of crescents from the fusion of two young ring-form parasites, as described and pictured by Mannaberg.

FIG. 9. A parasite attached to a corpuscular mound on the upper, external surface of the red corpuscle. A partial decolorization of the corpuscular mound can be seen.

FIG. 10. Complete decolorization, or dehemoglobinization, of the corpuscular area, which is encircled by the pseudopodium of the parasite.

FIG. 11. A peripheral corpuscular mound, encircled by two young parasites of varying sizes.

FIGS. 12 and 13. Parasites surrounding decolorized, or dehemoglobinized, corpuscular mounds. Here the nuclei conform to the contour of the mounds. These figures illustrate how much damage can be done to a corpuscle by a very young parasite, without any evidence of the presence of pigment in the protoplasm. That these corpuscular mounds are transparent, is shown by the fact that in Fig. 12 the periphery of the adjoining uninfected red corpuscle can be seen through the achromatic area, which overlies it; and in Fig. 13, the parasite underneath can be seen through the achromatic area of the overlying corpuscle.

These figures correspond with Mannaberg's and Marchiafava and Bignami's description and drawings of parasites resting in niches at the periphery of the red corpuscles.

FIG. 14. Two parasites attached to one peripheral corpuscular mound. A is on the under surface of the corpuscle, O on the upper surface. Note the difference in clearness and sharpness of outline between the two parasites.

FIG. 15. Two parasites, in similar stages of development, encircling a corpuscular mound at the periphery of the red corpuscle. The pseudopodium of the parasite on the left can be seen encircling the mound at O.

FIGS. 16 to 18. The gradual destruction, or decolorization, of the corpuscular mound encircled by parasites at opposite poles is seen.

FIG. 19. Two parasites occupying the one decolorized corpuscular mound.

FIG. 20. Two parasites, with bodies at opposite poles, occupying the one corpuscular mound.

FIG. 21. Two parasites, with bodies superimposed, occupying the one corpuscular mound.

FIG. 22. Parasites of varying sizes occupying the same corpuscular mound.

FIG. 23. Two parasites, in similar stages of development, occupying one corpuscular mound. At O the body of the lower parasite can be seen overlying that of the upper parasite.

FIG. 24. Three parasites, in similar stages of development, occupying one corpuscular mound. A careful examination of the picture will enable one to trace the outline of each parasite.

FIG. 25. Two parasites on a corpuscular mound near the periphery of the corpuscle. The body of the parasite at O extends beyond the periphery of the corpuscle and shows a granule of pigment in the protoplasm.

FIG. 26. Two parasites attached to the same peripheral corpuscular mound. One of the parasites is attached to the upper, the other to the under surface of the corpuscle.

FIGS. 27 and 28. Two parasites attached to one peripheral corpuscular mound.

FIG. 29. Three parasites attached to one peripheral corpuscular mound. At O one of the parasites is superimposed over the other.

FIGS. 30 and 31. Two parasites, one superimposed over the other, attached to the one corpuscular mound.

FIG. 32. Two parasites attached to the one decolorized corpuscular mound.

FIG. 33. Two parasites attached to the one corpuscular mound in such a manner that they resemble in shape a small crescent.

FIG. 34. Three young parasites attached to the one corpuscular mound.

PLATE 59.

TERTIAN PARASITES.

Magnification, × 1,900.

FIG. 1. Two young parasites, the result of a recent segmentation. The delicate attaching pseudopodium can be seen formed from the cytoplasm of the parasite and arising near the nucleus.

FIG. 2. A young parasite attached to a peripheral corpuscular mound.

FIG. 3. A young parasite attached to a hemoglobin mound on the upper surface of the corpuscle; the protoplasm of the parasite extends beyond the periphery of the corpuscle.

FIG. 4. A young parasite attached to a peripheral corpuscular mound.

FIG. 5. A young parasite attached to a decolorized peripheral corpuscular mound. There is no evidence of pigment in the protoplasm of the parasite.

FIG. 6. A free parasite, showing the pseudopodium but little extended.

FIG. 7. Two young parasites attached to one hemoglobin mound at the periphery of the red corpuscle. The nucleus of the upper parasite extends beyond the periphery of the corpuscle.

FIG. 8. Two parasites attached to a decolorized corpuscular mound.

FIG. 9. A ring-form parasite attached to the upper surface of the red corpuscle, the entire protoplasm of the parasite entering into the formation of the pseudopodium. The small achromatic area, which is parasitic, can be seen surrounding the chromatin substance.

FIG. 10. A parasite encircling a completely decolorized corpuscular area. A granule of pigment is seen in the protoplasm of the parasite.

FIG. 11. An adult, pigmented parasite attached to a peripheral corpuscular mound at the periphery of the red corpuscle at o. The pseudopodium of the parasite can be distinctly seen encircling the mound at its base.

FIG. 12. An adult, pigmented parasite attached to a peripheral corpuscular mound. Beginning decolorization of the corpuscles is seen at o.

FIGS. 13 to 17. Adult, pigmented parasites attached to corpuscular mounds at o. Fig. 15 shows a parasite with an accessory filament surrounding a secondary corpuscular mound at x.

FIG. 18. A parasite attached to a granular corpuscular skeleton, by means of its pseudopodium encircling a mound at o. The periphery of the corpuscular skeleton can be seen through the mound.

FIG. 19. Two young pigmented parasites attached to corpuscular mounds at o. The corpuscle shows Schuffner's granulation.

FIG. 20. Two adult, pigmented parasites attached to separate mounds, side by side, at oo.

FIGS. 21 to 24. Parasites attached to corpuscular mounds at o.

FIGS. 22, 23, and 24 show the mounds in various stages of destruction.

FIGS. 25 and 26. Two adult parasites attached to one corpuscular mound. These figures correspond to certain stages depicted by Ewing in his conjugation theory.

PLATE 60.

TERTIAN PARASITES.

Magnification, × 1,900.

FIG. 1. A young parasite encircling with its pseudopodium a completely decolorized corpuscular mound. The infected corpuscle shows Schuffner's granulation. This is evidence that Schuffner's granulation forms a part of the substance which is digested by the parasite.

FIG. 2. A parasite encircling an almost completely decolorized corpuscular mound, the corpuscle showing Schuffner's granulation.

FIG. 3. A young parasite encircling with its pseudopodium an almost completely decolorized corpuscular mound. This corpuscle shows blue granular stippling.

FIG. 4. A young parasite encircling a completely decolorized corpuscular mound. This corpuscle also shows blue granular stippling. The evidence here

suggests that the blue stippling also forms a part of the substance digested by the parasite.

Figs. 5, 6, and 7. Parasites surrounding corpuscular mounds on the upper surface of the red corpuscles. The area which belongs to the parasite and surrounds the nucleus is unstained and shows well against the hemoglobin of the corpuscles.

Fig. 8. An adult, pigmented parasite, whose pseudopodia can be seen arising from the cytoplasm of the parasite near its nucleus. They seem to end, one on either side of the decolorized corpuscular mound at the periphery of the corpuscle at o.

Figs. 9 and 10. The parasitic area surrounding the chromatin substance is stained pink, and contains no pigment granules.

Fig. 11. The parasitic area surrounding the chromatin granules is unstained. When this area is unstained and overlies a mound of corpuscular substance that has been completely decolorized by the action of the parasite, it may be impossible to differentiate one achromatic area from the other, thus giving the chromatin granules the false appearance of being entirely separated from the blue stained protoplasm of the parasite, and of belonging to the decolorized corpuscular area.

Fig. 12. A free, pigmented parasite, showing the achromatic area surrounding the chromatin granules.

Figs. 13 and 14. Two adult, pigmented parasites, on the periphery of the red corpuscles, the corpuscles showing evidence of degenerative changes due to the action of the parasites.

Fig. 15. An adult, pigmented parasite, attached to the upper surface of the red corpuscle. The nucleus of the parasite extends beyond the periphery of the infected corpuscle and overlies the adjoining red corpuscle at o.

Figs. 16 to 22. Free parasites with their pseudopodia in the form of loops, with the nuclei, with the exception of Fig. 16, at the extremity of the loop. These parasites have destroyed the corpuscles to which they were attached and are in migration, with the pseudopodia still in the form of the loops which had encircled the corpuscular mounds.

Fig. 23. A parasite attached to a corpuscular mound by a loop similar to those seen in connection with Figs. 17 to 22.

Plate 61.

Fig. 1. A free æstivo-autumnal parasite with loop attachment at o; also an æstivo-autumnal parasite with body and nucleus on the periphery of the red corpuscle. The pseudopodium is in the form of a loop, encircling a mound of unaltered hemoglobin which the parasite has pulled up for the purpose of attaching itself to it.

Figs. 2 to 4. The gradual decolorization, or dehemoglobinization, of that portion of the corpuscle which lies within the boundary of the encircling pseudopodium of the parasite. This condition results from the gradual digestion of the hemoglobin substance by the parasite.

Figs. 5 to 8. Æstivo-autumnal parasites attached in various positions to the surface of the red corpuscles by means of pseudopodia encircling hemoglobin

mounds at the periphery of the red corpuscles. These mounds have been pulled up by the parasites for the purpose of attachment and digestion.

FIG. 9. An æstivo-autumnal parasite attached to a hemoglobin mound at the periphery of the red corpuscle. This parasite can be seen on both surfaces of the red corpuscle; the part on the under surface can be seen at o.

FIG. 10. An æstivo-autumnal parasite attached to a corpuscular mound which has been completely decolorized, or dehemoglobinized, by the action of the parasite.

FIG. 11. Two æstivo-autumnal parasites attached to the same hemoglobin mound at the periphery of the red corpuscle, their pseudopodia encircling the mound at its base. The parasite on the right is on the upper surface of the corpuscle; the parasite on the left is on the under surface and, being seen through the substance of the corpuscle, shows less distinctly than the one on the right.

FIG. 12. An æstivo-autumnal parasite attached to the upper surface of the red corpuscle by means of its pseudopodium encircling a hemoglobin mound, which has been pulled up by the parasite. The mound can be seen to be pressed down on the surface of the corpuscle.

FIGS. 13 and 14. Two æstivo-autumnal parasites attached to the upper surface of the red corpuscle by means of pseudopodia encircling one hemoglobin mound. These two figures correspond to the parasites described by Craig as "conjugating young parasites."

FIG. 15. Two young æstivo-autumnal parasites attached, side by side, to separate decolorized corpuscular mounds. These parasites correspond to the parasites described by Mannaberg as "paired parasites," which he believed coalesced to form a crescent.

FIG. 16. Two young æstivo-autumnal parasites attached to one decolorized corpuscular mound, resembling, in this relation to each other, a small crescent.

FIG. 17. Two young tertian parasites, one slightly thicker than the other, attached side by side to separate hemoglobin mounds, the periphery of one ring overlying that of the other ring. These parasites correspond to the parasites described by Ewing as "conjugating rings of unequal size."

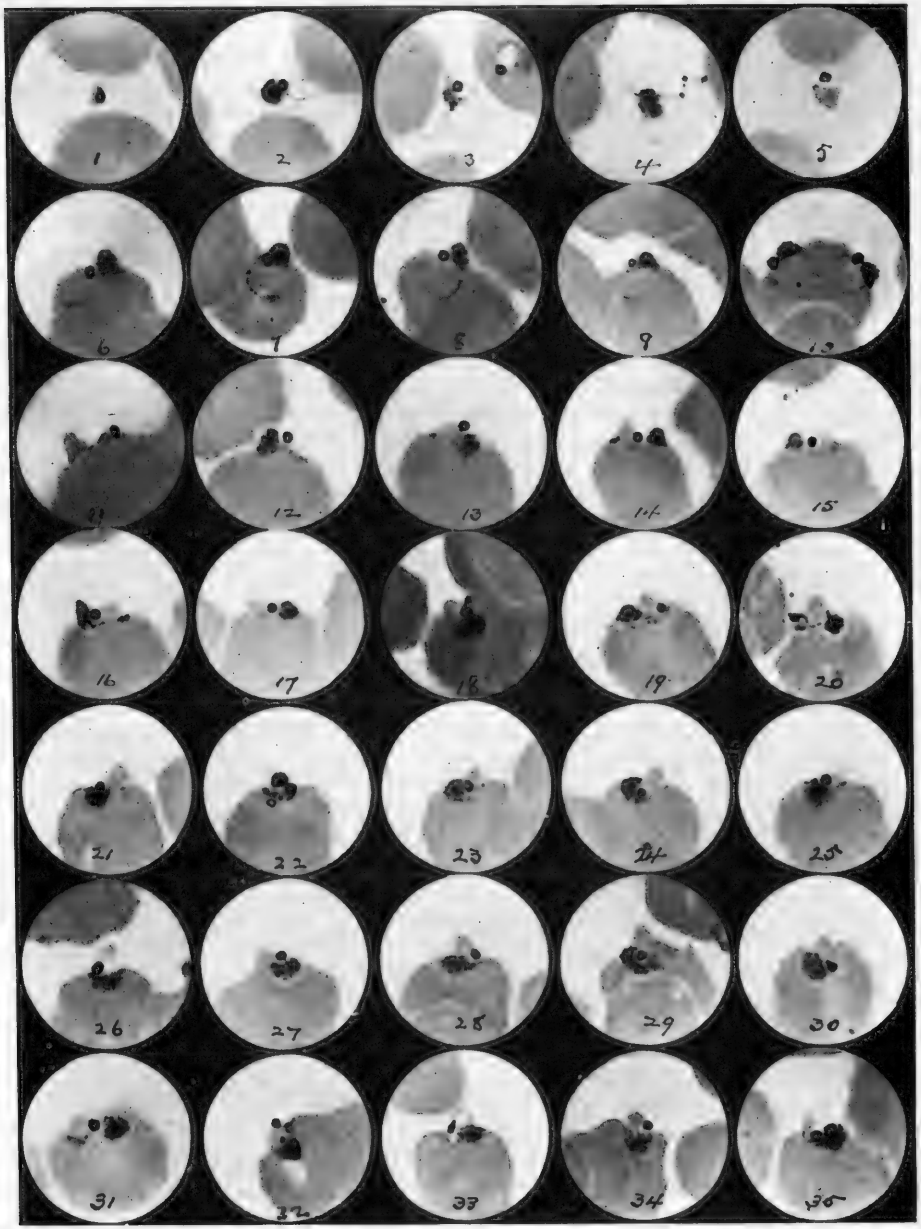
FIG. 18. Two adult, pigmented, tertian parasites, attached to one decolorized corpuscular mound. A slight separation can be seen between the two groups of chromatin granules. The accessory pseudopodia of these parasites are superimposed while surrounding a secondary hemoglobin mound at o. These parasites correspond to the parasites described by Ewing as the result of "a complete union of bodies and nuclei."

FIG. 19. An adult, tertian parasite surrounding with its pseudopodium a decolorized corpuscular mound. The chromatin mass is at the extremity of the loop.

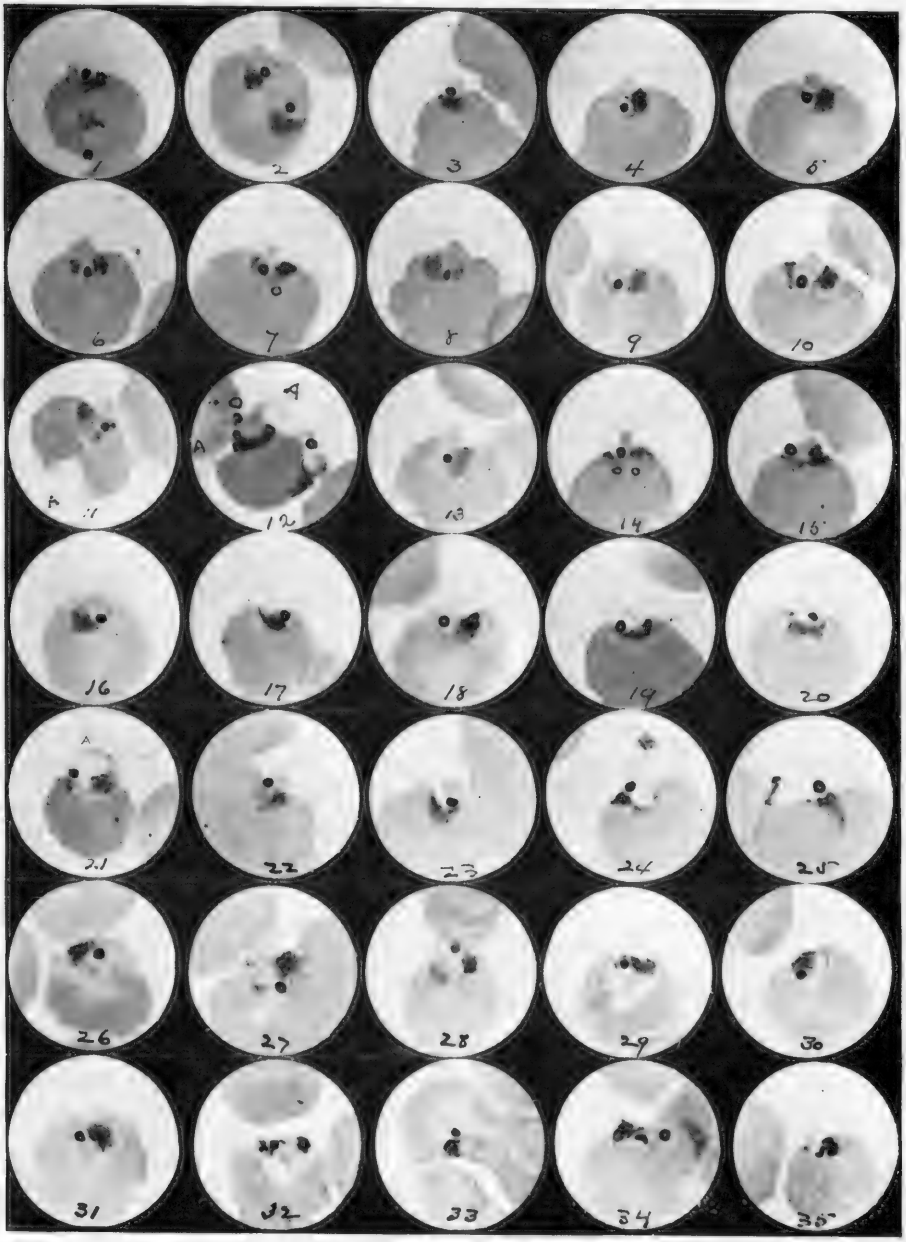
FIG. 20. A free tertian parasite, showing the pseudopodium in the form of a loop with the chromatin mass at the extremity of the loop.

FIG. 21. A tertian parasite showing the pink stained area surrounding the chromatin granules. The red corpuscle shows Schuffner's granulation.

FIG. 22. A tertian parasite, showing the blue stained area surrounding the chromatin granules. The corpuscular mounds to which the parasite is attached are decolorized by the action of the parasite.



(Lawson: Relation of Malarial Parasite.)



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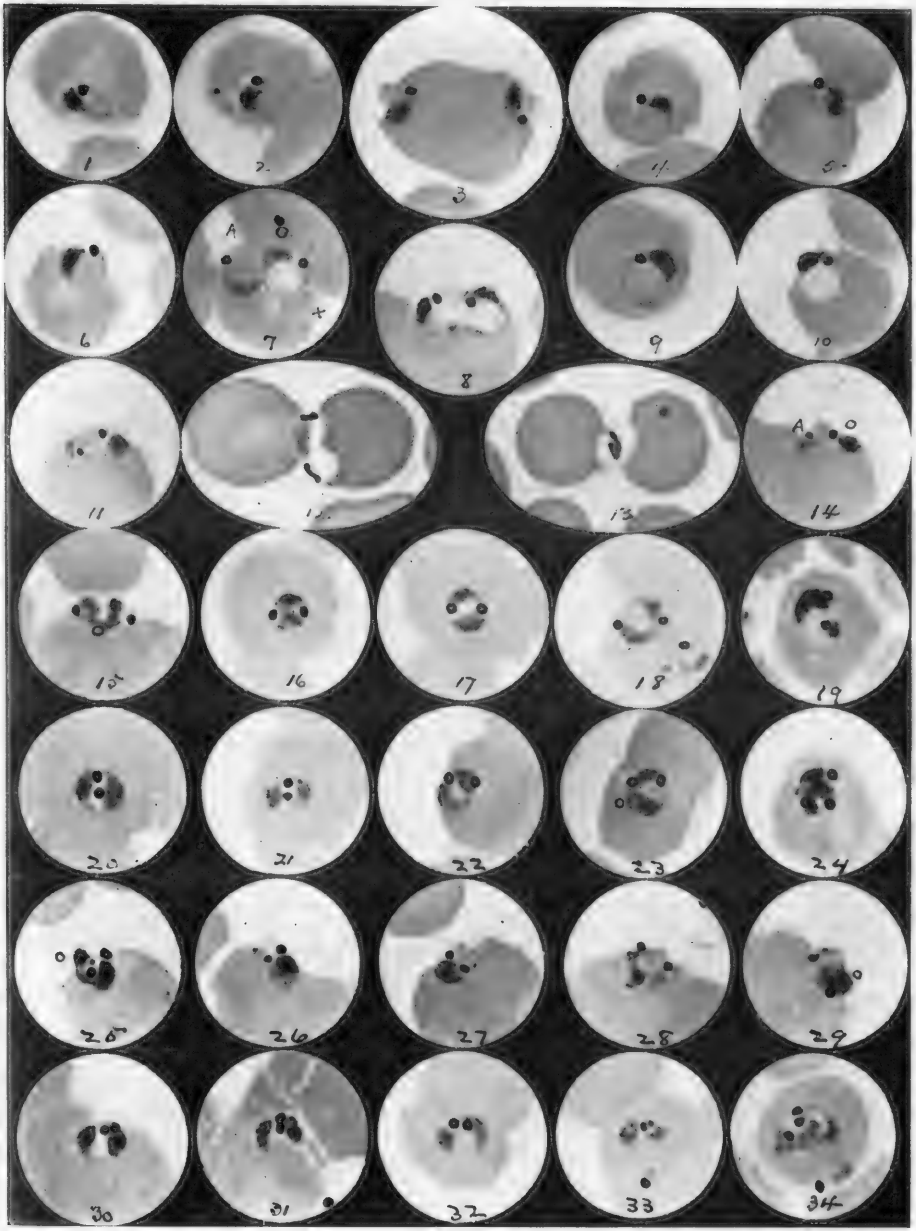
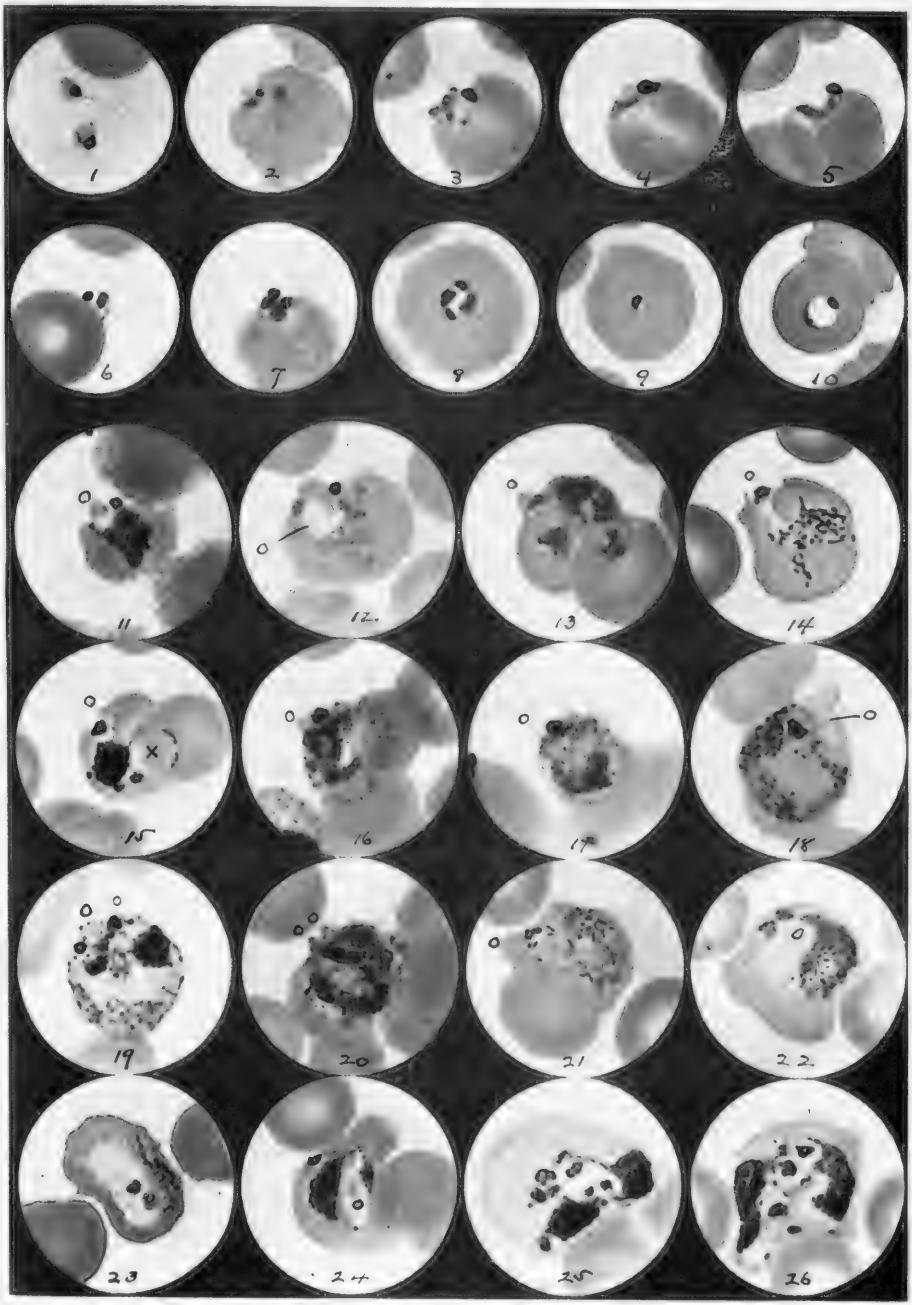
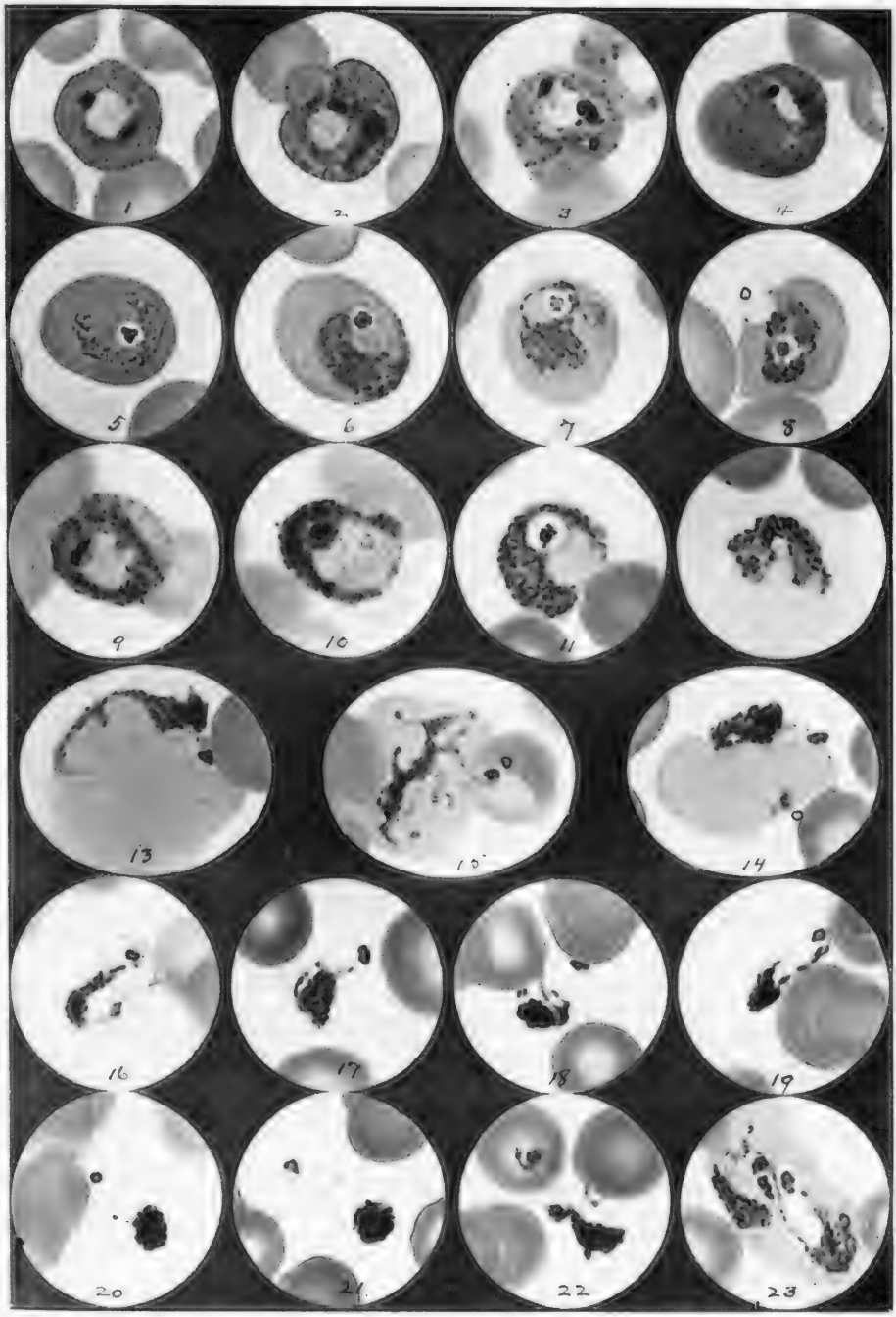


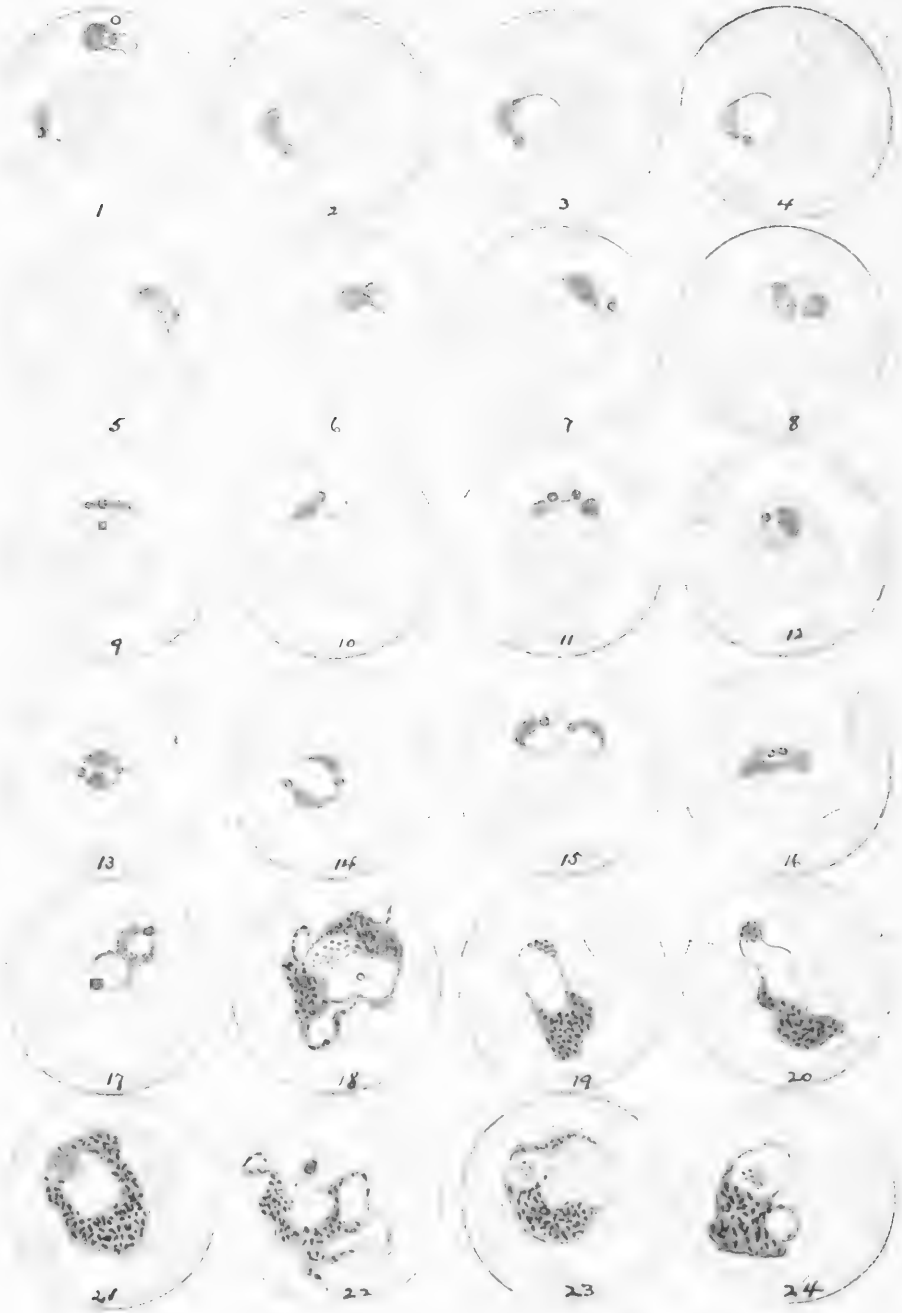
(Lawson : Relation of Malarial Parasite.)



(Lawson: Relation of Malarial Parasite.)



(Lawson : Relation of Malarial Parasite.)



(Lawson : Relation of Malarial Parasite.)

FIG. 23. A tertian parasite with an unstained area surrounding the chromatin granules. The red corpuscle shows Schuffner's granulation.

FIG. 24. A tertian parasite with an unstained area which is a part of the parasite, and which surrounds the chromatin granules, and overlies a mound of corpuscular substance that has been completely decolorized by the action of the parasite. It may be impossible to differentiate one achromatic area from the other. This appearance might lead an observer to believe the entire decolorized area to be parasitic.

THE INFLUENCE OF LECITHIN AND CHOLESTERIN UPON THE GROWTH OF TUMORS.*

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In view of the marked influence of lecithin upon normal growth[1] we undertook to determine the influence of lecithin upon the growth of tumors; and since, in most instances, cholesterin acts in an antagonistic sense to lecithin, we decided to determine also the influence of cholesterin upon tumor growth.

METHODS.

Lecithin.—The lecithin employed was prepared in the following manner: The yolks of eggs were carefully washed in running water to free them from adherent whites. They were then broken and mixed, and an equal volume of a 10 per cent. solution of sodium chloride was added to them. The solution thus obtained was shaken up with twice its volume of ether until the mixture attained a thick oily consistency. It was then put aside over night in separatory funnels. In the morning the mixture had separated into sharply defined layers. The ether layer was removed and evaporated at room temperature to about one sixth of its volume, the lecithin was then precipitated by adding to this concentrated extract four volumes of acetone. The precipitate was allowed to settle in a tall glass cylinder, the greater part of the supernatant fluid was decanted, and the thick subnatant suspension of lecithin was collected upon a hardened filter. After washing this precipitate several times with acetone and allowing it to drain thoroughly, it was scraped off the paper into a glazed porcelain dish and dried over sulphuric acid in an incubator at 36° C. for over a week. The lecithin was then kept during the course of the experiment in a desiccator over sulphuric acid at room temperature. The product was a light brown mass which could be crumbled with difficulty and became somewhat pasty in consistency at temperatures of about 40° C.

In preparing the lecithin for injection into animals, a weighed amount was placed in a porcelain mortar and a corresponding volume of M/6 sodium chloride solution was heated nearly to boiling and a few cubic centimeters were poured

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[1] Danilewsky, B., *Compt. rend Acad. de sc.*, 1895, cxxi, 1167; King, H. D., *Biol. Bull.*, 1907, xiii, 40; Johnson, M. E., *University of California Publications in Zoölogy*, 1912, xi, 54.

into the mortar. The lecithin was softened by the hot water and could readily be macerated until it formed a thick pasty mass with the sodium chloride solution. The remainder of the sodium chloride solution, which had been allowed to cool to about 70° C., was then added little by little, and the mass was triturated thoroughly so as to obtain a uniform mixture. In this way fine and very stable emulsions of lecithin were readily prepared. They could be coagulated by boiling for several minutes or by adding salts of the alkaline earths. They were, however, employed for injections without further treatment.

Cholesterin.[2]—The usual difficulty was experienced in making stable suspensions. In the preliminary experiments we dissolved the cholesterin in a little hot alcohol and poured this solution into water. The suspensions thus formed, however, are not stable and contain numerous small crystals which block the hypodermic needle and render injection very difficult. Later it was found that acetone was much more suitable for this purpose than alcohol, and it had the further advantage that it could be readily driven off from the mixture by heating, and without injuring the stability of the emulsion. Such an emulsion does not deposit cholesterin even after standing at room temperature for over a month. Before this was ascertained, however, we found that very satisfactory emulsions could be obtained by triturating cholesterin in N/10 sodium oleate solution, and these were the emulsions employed throughout the second series of experiments. The weighed cholesterin was placed in a mortar, the sodium oleate solution was heated to boiling, and was then added little by little to the cholesterin, the whole mixture being carefully macerated and triturated to secure a uniform mixture. The emulsion thus obtained was heated just to boiling in an Erlenmeyer flask plugged with cotton, and was allowed to cool without removing the plug. Samples were withdrawn from time to time for use as needed.

HISTORY OF THE TUMORS.

We propagated the Flexner-Jobling carcinoma[3] by inoculation into the axillary region through four successive series of white rats, during which process the virulence of the tumor greatly decreased, the percentage of successful inoculations falling from 60 per cent. in the first series to 20 per cent. in the fourth series. The rats employed varied in age from half grown to adult, and were all obtained from local sources.

Preliminary Series.—In a preliminary series of experiments 101 white rats, obtained from local sources, were inoculated in the axillary region with portions of a tumor taken from the fourth series

[2] The cholesterin employed was Gruebler's purissimum crystallized or Kahlbaum's crystallized.

[3] Through the courtesy of Dr. Peyton Rous, of The Rockefeller Institute for Medical Research, we obtained in March of this year two specimens of the Flexner-Jobling carcinoma in white rats.

above mentioned. In all, there were twenty-four successful inoculations. These tumors grew extremely slowly, and of six which were kept as controls and not treated in any way, three underwent spontaneous degeneration and completely disappeared. The remaining three attained the average diameter of eleven millimeters only after five weeks of growth.

As our tumor appeared to have lost its virulence, the results obtained with these tumors, although confirmatory of the results subsequently obtained, were regarded as inconclusive.

For the second series of inoculations we therefore obtained two more specimens of the Flexner-Jobling tumor from the same source.

Second Series.—Sixty-one white rats were inoculated in the axillary region with portions of one of these tumors. The number of successful inoculations was 42, or 69 per cent.

At the same time sixty-four rats obtained from dealers in Chicago were similarly inoculated with portions of one of the control tumors from the preliminary experiment. The number of successful inoculations was 55, or 86 per cent.

Both sets of tumors grew rapidly, attaining average diameters of 15 and 11.9 millimeters respectively after nineteen days. The tumors obtained by inoculating local rats with the tumor supplied to us by Dr. Rous are designated as "local," while those which were obtained by inoculating rats obtained from Chicago with the tumor propagated here through five series of rats are designated "Chicago."

RESULTS.

In the preliminary experiments, as already stated, twenty-four animals with tumors were employed. The injections were started twenty days after inoculation. No accurate measurements of the tumors were made, but when the injections were begun their average estimated diameter lay between eight and eleven millimeters. Eleven animals received lecithin upon the 20th, 23d, 27th, 30th, 35th, 39th, 42d, and 46th days following the inoculation of the tumor, one cubic centimeter of 1 per cent. lecithin emulsion being injected directly into the tumors. Seven animals received cholesterin upon the twentieth and twenty-third days following the

inoculation, one cubic centimeter of 1 per cent. alcohol-cholesterin suspension being injected directly into the tumors. Six animals were kept as controls.

Even three days after the first injection it was noticed that the growth of the tumors in the animals that had received cholesterin had undergone a remarkable acceleration. Within a week they were double the size of the tumors in the control animals, and they maintained this lead throughout the experiment. Two spontaneous cures occurred after the forty-second day, whereas three of the tumors in the control animals underwent degeneration and had disappeared by the thirty-fifth day after inoculation.

The tumors in the animals treated with lecithin, on the contrary, grew more slowly than those in the normal animals and exhibited a greater proportion of spontaneous cures, for eight of the eleven tumors had degenerated and completely disappeared by the fiftieth day. On the fiftieth day the animals which still had tumors received two cubic centimeters of 2 per cent. lecithin emulsion. On the fifty-sixth day the average diameter of these tumors was twenty-two millimeters, while that of the tumor in the one survivor among the rats which had been treated with cholesterin was forty-four millimeters. After the fifty-sixth day the experiment was discontinued.

These results, although indicating a marked acceleration of tumor growth after the administration of cholesterin, and a possible retardation after the administration of lecithin, were inconclusive. The second series of experiments was therefore undertaken. The results of this series were decisive, and confirmed the tentative conclusions reached in the course of the preliminary experiments.

TABLE I.

Source of animal.	No. of controls.	No. treated with	
		1 c.c. of 2 per cent. lecithin emulsion.	1 c.c. of 3.9 per cent. cholesterin emulsion in N/10 sodium oleate.
Local	12	20	10
Chicago	12	30	13
Totals	24	50	23

The days on which injections of lecithin and cholesterin were given are shown in table II.

As already stated, ninety-seven animals ("local" and "Chicago") were inoculated with two tumors having different histories. No spontaneous cures occurred in any of the animals, and the tumors grew rapidly. The animals were apportioned without selection as shown in table I.

TABLE II.

Source of animal.	Days after inoculation.								
Local.....	19	21	24	26	28	31	33	35	38
Chicago.....	18	20	23	25	27	30	32	34	37

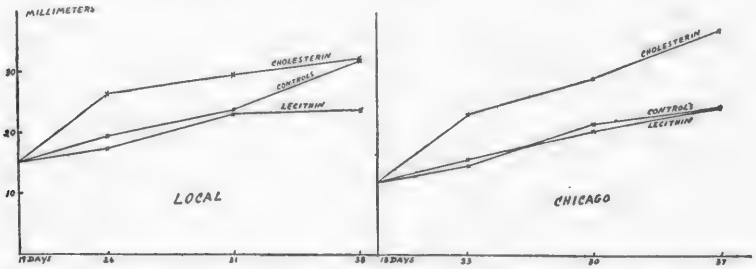
The tumors were measured through the skin in two diameters at right angles to each other on the 19th (18th), 24th (23d), 31st (30th), and 38th (37th) days after inoculation. When the tumors were small, the measurements were made with a pair of callipers. The mean of the longest and shortest diameters was recorded as the average diameter of any given tumor, and the average of these estimates gives the average diameter of the tumors in any given group of animals. This method of measurement was found to yield constant results, any ten unselected animals from either the local or the Chicago groups yielding, on the first day of measurement, the same figures for the average diameter within two or three millimeters. Consequently the average diameter of the tumors in the lecithin or cholesterin groups was considered to be that of the tumors in all the animals from the same source.

On the 24th (23d) day the lecithin animals were divided into two subgroups. On that day and thenceforward one half of these animals received lecithin suspended in M/6 sodium chloride and the other half received the same amount of lecithin suspended, however, in M/6 strontium chloride. The latter suspension was prepared by first suspending the lecithin in distilled water, and then adding sufficient concentrated strontium chloride solution to bring the final concentration up to M/6. The addition of the strontium chloride caused the lecithin to coagulate and form flocculi, which, however, did not prevent uniform mixture just before each injection, or impede the injection itself. Since strontium chloride renders lecithin insoluble, we employed a suspension of lecithin in strontium chloride solution, thinking that possibly lecithin injected

together with strontium chloride might stay longer within the tumors and thus exert its action, at least locally, for a longer period.

All the injections were made directly into the tumors.

The results obtained are given in table III and are also shown in text-figure 1.



TEXT-FIG. 1. Comparative growth of tumors treated with lecithin and cholesterin and untreated.

TABLE III.

Local Animals.

Treatment.	Average diameter of tumors in mm. after			
	19 days.	24 days.	31 days.	38 days.
Controls	15.0	19.8	24.0	31.9
Lecithin in M/6 sodium chloride	——	——	24.1	23.5
Lecithin in M/6 strontium chloride	——	——	22.6	24.5
Lecithin (inclusive)	15.0	17.6	23.4	24.0
Cholesterin	15.0	26.6	29.7	32.3

Chicago Animals.

Treatment.	Average diameter of tumors in mm. after			
	18 days.	23 days.	30 days.	37 days.
Controls	11.9	14.8	21.7	24.7
Lecithin in M/6 sodium chloride	——	——	20.0	24.0
Lecithin in M/6 strontium chloride	——	——	21.8	25.3
Lecithin (inclusive)	11.9	15.8	20.9	24.7
Cholesterin	11.9	23.3	29.2	37.2

It will be seen that the growth of the tumors in each group of animals was decidedly accelerated by the injections of cholesterin. This acceleration was, however, much more marked, especially in the local animals, in the first few days after the beginning of the treatments than it was later on, so that in the case of the local

animals, by the thirty-eighth day the size of the tumors in the controls almost equaled the size of the tumors which had been treated with cholesterin. On the other hand, scarcely any retarding action of lecithin is evident in the earlier stages of growth, but between the thirty-first and thirty-eighth days in the local animals the retardation due to lecithin was very evident. Table IV shows the comparative increase in growth.

TABLE IV.

Source of animal.	Treatment.	Increase in diameter in mm. between the		
		19th and 24th days.	24th and 31st days.	31st and 38th days.
Local.....	Controls	4.8	4.2	7.9
	Lecithin	2.6	5.8	0.6
	Cholesterin	11.6	3.1	2.6
Chicago.....	Controls	2.9	6.9	3.0
	Lecithin	3.9	5.1	3.8
	Cholesterin	11.4	5.9	8.0

No retardation of tumor growth due to lecithin is shown by the figures for the Chicago animals. As we shall see, however, the lecithin retarded markedly the growth of metastases in these animals, so that if we add metastatic to primary tumor growth the results obtained with these two groups of animals are identical in character.

It is evident from table III that the simultaneous administration of strontium chloride did not affect the action of lecithin.

Between the 31st (30th) and 38th (37th) days, four animals died in the Chicago lecithin group, one in the Chicago lecithin strontium chloride group, and one in the local cholesterin group. The tumors in these animals were measured on the days upon which death occurred, and were included in the estimates of the average diameters upon the 38th (37th) day. It was evident, however, from the poor condition of several of the animals, that more deaths might occur shortly and thus render comparison of the groups increasingly difficult and inaccurate.[4] Moreover, several animals had developed sinuses within the tumors and it was almost impossible

[4] Most of the animals that died or were in poor condition were found, upon post-mortem examination, to have pneumonic patches in the lungs.

to secure retention of the injected fluids within such tumors. Accordingly the experiment was terminated at this point, and a post-mortem examination of all the animals was carried out. Metastases were found in the lungs of many of the animals.[5] In table V the post-mortem findings are given.

TABLE V.

Source of animal.	Treatment.	No. of animals examined.	Broken down (scars opening upon the surface).		Metastases.	
			No.	Per cent.	No.	Per cent.
Local.....	Controls	11	3	27	3	27
	Lecithin	19	16	84	4	21
	Cholesterin	9	3	33	2	22
Chicago.....	Controls	8	0	0	4	50
	Lecithin	29	11	38	7	24
	Cholesterin	13	11	85	6	46

It is evident that in the Chicago animals lecithin greatly decreased the tendency to form metastases. This was evident in both groups of animals, but it was especially striking when the number and size of the metastases in the animals treated with lecithin were compared with those in the other animals.

When metastases were found in animals that had received lecithin they were small and few in number, usually consisting of one or two isolated spots in the lungs, while in all the animals treated with cholesterin in which metastases were found, and in three of the Chicago controls, the metastases formed dense masses throughout the greater part of the lung tissue. It may therefore be concluded that cholesterin greatly increases the extent of metastatic growth, while lecithin retards it.

Since in the animals treated with lecithin and in the local controls the metastases were few in number and small in extent on the thirty-eighth day, and since retardation of the primary tumor growth in the local animals was evident only between the thirty-first and thirty-eighth days, it would appear that the retarding action of lecithin occurs chiefly at a period of the growth of the

[5] We are indebted to Drs. F. P. Gay and G. Y. Rusk for examining these growths and confirming their metastatic nature.

tumors which roughly corresponds with the beginning of the metastatic stage.

CONCLUSIONS.

1. Cholesterin, whether suspended in dilute alcohol or in sodium oleate solution, when injected directly into tumors causes a marked acceleration both of the primary and of the metastatic growth.

2. The acceleration of the growth of the primary tumor by cholesterin is most evident in the premetastatic stage.

3. Lecithin, when injected in the form of an aqueous emulsion directly into tumors, diminishes the tendency to form metastases, retards the metastatic growth when it does occur, and in some instances also retards the primary growth.

4. The retardation due to lecithin is most evident in the metastatic stage.

5. Simultaneous injection of M/6 strontium chloride solution into the tumors does not appreciably affect the action of the lecithin.

PNEUMONIC LESIONS MADE BY INTRABRONCHIAL INSUFFLATION OF NON-VIRULENT PNEUMOCOCCI.*

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INTRODUCTION.

In two series of investigations pneumonia has been produced experimentally in dogs by means of intrabronchial insufflation of virulent organisms. Lamar and Meltzer[1] produced lobar pneumonia in a large number of animals by insufflation of cultures of a highly virulent pneumococcus, and in a smaller number by the insufflation of *Streptococcus mucosus* and of Friedländer's pneumobacillus. We[2] produced lobular pneumonia in twenty dogs by the insufflation of cultures of streptococcus, and in eleven dogs by the insufflation of cultures of the influenza bacillus. These experimental results agree with the pathological experience with human beings; in lobar pneumonia it is the pneumococcus which is found in the exudate, and in bronchopneumonia the streptococcus is more often present. Both forms of experimental pneumonia have, of course, essential features in common; namely, there is in both an acute exudative inflammation, the exudate containing polymorphonuclear leucocytes, serum, and desquamated epithelial cells. Later large mononuclear phagocytes and cell debris also appear in both. But the pneumonias have also some features which sharply distinguish one from the other. We discussed these distinguishing features in our last paper.[3] For the purpose of our present paper it is well to enumerate them again. However, we wish to say expressly that these features have reference only to the experimental

* Received for publication, December 10, 1912.

[1] Lamar, R. V., and Meltzer, S. J., *Jour. Exper. Med.*, 1912, xv, 133.

[2] Wollstein, M., and Meltzer, S. J., *Jour. Exper. Med.*, 1912, xvi, 126.

[3] Wollstein, M., and Meltzer, S. J., *loc. cit.*

pneumonias produced by the method of intrabronchial insufflation, and only as we know them at present.

1. In experimental lobar pneumonia there was a mortality of 16 per cent.; in experimental bronchopneumonia there was no mortality. We should, however, add that in this respect the two series of experiments were not sufficienty comparable to permit of a final conclusion. Not only was the number of experiments with the streptococcus only about one half as large as the number of those of Lamar and Meltzer with the pneumococcus, but most of the animals were killed at too early a stage after the inoculation to permit drawing a definite conclusion as to the actual mortality of this infection. For the present we have to be satisfied with the statement that in none of our uncomplicated cases of experimental bronchopneumonia was there a fatal tendency of the disease. Furthermore in the experiments with the pneumococcus the course seemed to depend upon the quantity of injected culture ("most of the deaths followed the injection of a large quantity of culture, and, conversely, the injection of a large quantity was usually fatal"),[4] while in our experiments with the streptococcus large doses did not seem to affect the final outcome. For instance, in an animal which received thirty cubic centimeters of culture and which was killed on the sixth day, the animal was not sicker than other animals that received fifteen or ten cubic centimeters of the culture, nor was the resolution of the exudate retarded.

2. In the pneumococcus inoculations a pneumococcus septicemia developed in all the fatal cases; no bacteremia occurred in the streptococcus cases.

3. In the pneumococcus lesions the consolidated part of the lung was always dense and air-free, *i. e.*, lobar in character, no matter how large or small the affected area was; in the lesions produced by the streptococcus, no matter how large the area or how intense the inflammatory process was, aerated lobules were always discernible among the solid foci; *i. e.*, the streptococcus lesion was lobular.

4. In pneumococcus pneumonia there was always a definite pleurisy present even in all the non-fatal cases, while it was practically absent in all the streptococcus infections.

[4] Lamar, R. V., and Meltzer, S. J., *loc. cit.*, p. 141.

5. In the pneumococcus lesions the cut surface was rather dry and granular; in the lesion produced by the streptococcus the cut surface was moist and smooth.

6. Fibrin was an important element in the exudate of the lesions brought about by the pneumococcus, while it played practically no part in the streptococcus pneumonia.

7. In the pneumococcus lesions the walls of the alveoli and bronchioles remained free from infiltration although their lumina may have been densely packed with exudate; on the other hand, in the lobular pneumonia produced by the streptococcus not only the walls of the alveoli and bronchioles but also the connective tissue septa and the adventitia of the blood vessels were the seat of marked infiltration.

8. The resolution of the pneumonic lesions set in and terminated earlier in the consolidations from streptococci than in those caused by pneumococci.

We may now add a further point of differentiation. In the course of further study we made many experiments with insufflation of virulent pneumococci. We found that in every case twenty-four hours after insufflation of a virulent pneumococcus this organism could be cultivated from the heart's blood, whereas three or four days later in the non-fatal cases the lungs as well as the blood were free from the pneumococcus. This is an interesting point and *probably has its analogy in clinical findings.* On the other hand, in pneumonia produced by the insufflation of streptococcus the blood remained free from the infecting organism. We may therefore state:

9. Insufflation of virulent pneumococci leads in all cases to bacteremia at the end of the first day after inoculation. The bacteremia disappears in the non-fatal cases, while after insufflation of streptococci no bacteremia develops.

From the above it seems that in general the inoculation of the streptococcus produced a milder effect than the pneumococcus. In discussing the results of both series of investigations, we said that since different organisms introduced in the same way and under conditions which are apparently the same produce distinctly different pneumonic lesions in animals of the same species, the con-

clusion presents itself that different types of pneumonia are produced by specifically different bacteria. We added, however, that further investigation may show that the differences in the nature of the lesions are due rather to the degree of virulence of the causative microörganism than to differences in the species, that even apperently virulent streptococci may nevertheless be less virulent than certain virulent pneumococci, and that such different lesions as were here obtained by different organisms may perhaps be obtained by organisms of the same species possessing different degrees of virulence.

This point has now been tested in a series of experiments on dogs with the intrabronchial insufflation of a non-virulent strain of pneumococcus. The non-virulent strain was obtained from Dr. Dochez of the Rockefeller Institute Hospital, by whom it was isolated from the blood of a patient with lobar pneumonia who recovered. The microörganism[5] had the morphological and cultural features of the pneumococcus, but failed to kill a mouse in doses less than one half cubic centimeter of a twenty-four hour broth culture when freshly isolated from the patient's blood. After cultivation in the laboratory it failed to kill in any dose.

EXPERIMENTAL DATA.

Twenty to twenty-four hour broth cultures of the avirulent pneumococcus were injected by intrabronchial insufflation into eighteen dogs, in doses varying from ten to thirty cubic centimeters. In three instances the dose of cocci was enriched three times; that is, it contained three times the number of organisms, as the result of centrifugalization of a large quantity of culture. The method of injection by intrabronchial insufflation has been described in detail by Lamar and Meltzer,[6] and consists essentially of the introduction of a tube through the mouth, larynx, and trachea deeply into a

[5] This strain of pneumococcus was soluble in rabbit bile, though it was avirulent for white mice, and, as will be shown later, it differed from the virulent pneumococcus in its effect on the lungs of dogs. This solubility of an avirulent pneumococcus is contrary to the statement of Neufeld and Haendel (Kolle, W., and Wasserman, A., Handbuch der pathogenen Mikroorganismen, Jena, 1912, iv, 527) that avirulent strains are not dissolved in bile.

[6] Lamar, R. V., and Meltzer, S. J., *loc. cit.*

bronchus, the culture being introduced through the tube. Usually the tube slips into the right bronchus.

None of the dogs died as the result of the inoculation of these doses of avirulent pneumococci. All were killed at periods varying from one to seven days after the dose had been administered. The animals did not appear to be very ill, and only three showed a rise of temperature to 41.1° C., all three having received a dose of ten cubic centimeters. The animals receiving the enriched doses, as well as one dog to whom thirty cubic centimeters were given, had even less fever and no more severe local reaction than the dogs to whom the smaller dose had been administered.

The Lesions in the Lungs.—The gross appearance of the lesions in the lungs of the dogs varied according to the length of time elapsing between the injection and the killing of the animal; but in parellel stages the differences were very slight. The size of the dose influenced the course of the disease and the severity of the lesion but little, a point in marked contrast to the experience of Lamar and Meltzer with virulent pneumococci. There was no exudate on the surface of the pleura in any one of the eighteen cases, but a lack of luster was noted in three instances, one and two days after inoculation. In twenty-four hours after insufflation of a dose of ten or fifteen cubic centimeters of the avirulent organisms, the whole or part of one lobe was the seat of hepatization. In two cases the right posterior lobe, in two cases the left posterior lobe, and in one case the right anterior lobe was involved. In two cases the entire lobe was solid, in two others only two thirds of the lung was involved, and in one all but the superior anterior angle of the lobe was solid. The lobe was heavy, firm, but not much larger than its uninvolved neighbors. On section the hepatized lung was red, moist, and evenly consolidated; only blood could be expressed. From the bronchi frothy fluid ran, thin, not viscid. Aerated lobules were not seen within the solid areas, but at their periphery, the line of demarcation being irregular. Pneumococci were found in small numbers in smears made from the lung, and they grew sparingly in cultures; the heart blood was sterile. The anatomical diagnosis was that of an early, not severe lobar pneumonia in the stage of red hepatization, the condition of engorgement being still present in portions of the lobe.

In forty-eight hours the lesion had become more marked; hepatization was more advanced, though no larger area of lung was involved. Thus the left posterior lobe except its inferior border was solid in one case, three quarters of the right posterior in another, two thirds of the left anterior and the left posterior in another, and two thirds of the right posterior with a small area in the subcardiac lobe in still another animal. These lungs were more solid and heavy than those seen on the earlier day; on section they were mottled red and gray, smoothly consolidated, friable, and less moist. It was difficult to find pneumococci in smears, and none grew in cultures.

Microscopic examination after twenty-four hours showed that the alveoli were filled with polymorphonuclear leucocytes in large numbers, very few epithelial cells, and little fibrin. The capillaries in the alveolar walls were distended with blood, but the walls of the alveoli were not infiltrated with cells nor with fibrin. The congestion was most marked just beneath the pleura, and some of the most solidly packed areas were also there. The alveoli were not equally filled with exudate, some containing only fibrin and a few desquamated epithelial cells, others a few red cells in addition to these, and in others the leucocytes were most abundant, nuclear fragmentation being well under way. The smallest bronchi contained plugs of fibrin and leucocytes, while their walls showed leucocytic infiltration; the larger bronchi, on the other hand, were quite normal, showing neither desquamation of their epithelia nor infiltration of their walls; they were empty. Some medium sized bronchi contained masses of fibrin, leucocytes, and red cells quite detached from their lining cells, evidently sputum in the course of expectoration. Pneumococci were few in number in the alveoli and in the bronchi. Areas of leucocytic exudation about small veins were numerous.

In forty-eight hours the exudate was found to have become more leucocytic in character, the epithelial cells being few in number and the red blood cells only occasional. The amount of fibrin had not increased. The most solid areas were again found beneath the pleura. The bronchi showed the same lesions as in the earlier stage, the infiltration of their walls not being more marked or more general.

Five days after insufflation of a dose of ten cubic centimeters resolution was going on in a large portion of the right posterior lobe, though a small area of pneumonia still remained. In six days there were no solidly packed alveoli remaining, and resolution was almost complete.

A larger dose of twenty cubic centimeters had caused a lobar pneumonia still at its height on the third day, with an exudate consisting mostly of polynuclear leucocytes and with little fibrin. That the disease was still progressing on the third day was evidenced in the animal by the fact that while the right posterior lobe showed the lesion of red hepatization, the upper half of the left posterior lobe showed an earlier stage of engorgement and edema, with an exudation of serum, epithelial cells, red blood cells in small numbers, and less fibrin than was present in the later stage. This left posterior lobe was not swollen, but dark, firm, heavier than its aerated neighbor, and its section was moist, not crepitant, but not entirely airless.

The pneumonia caused by a dose of twenty cubic centimeters was quite resolved on the sixth and seventh days, leaving only a dark, congested, inelastic area to indicate grossly where the lesion had been. On microscopic examination there was evidence of phagocytosis of cellular fragments and the presence of granular, poorly staining debris in some alveoli.

In a dog that received thirty cubic centimeters the lungs presented on the third day a well marked but not intense lobar pneumonia (red hepatization) in the upper half of the right posterior lobe. The amount of fibrin present in the alveolar exudate was only moderate, leucocytes being very numerous.

An enriched dose, containing in fifteen cubic centimeters of broth the cocci grown in forty-five cubic centimeters, produced a lesion in twenty-four hours which occupied the right posterior lobe except its anterior border; the lobe was red, solid, not friable, evenly consolidated, moist, and without pleurisy. Its exudate consisted of polymorphonuclear leucocytes, few epithelial cells, and very little fibrin contained in alveoli whose walls were quite free from infiltration of any kind. The lesion was not more severe than one produced by a dose of fifteen cubic centimeters. Another

dog, after an enriched dose similar to the above, showed a resolving lobar pneumonia on the third day, about two thirds of the left posterior lobe having been involved. On microscopic examination the extent of the resolution was surprisingly far advanced, considering the size of the dose of cocci administered.

DISCUSSION.

The experiments established, in the first place, the fact that the insufflation of a culture of avirulent pneumococcus invariably caused the development of a pulmonary lesion which macroscopically had the appearance of a lobar pneumonia. The consolidated part was firm, solid, heavy, and contained no aerated lobules in its midst. The inflammation, however, was less intense than it usually is in the lesions of lobar pneumonia caused by virulent pneumococci, the progress was generally slower, and the resolution set in earlier. Furthermore, in none of the animals had the disease a fatal termination, and the increase of the quantity of the insufflated culture had not the same effect upon the course of the inflammatory process that it undoubtedly had in the experiments with the virulent pneumococcus. Still more important perhaps are the following differences. The exudate produced by the avirulent pneumococcus contained strikingly less fibrin than the exudates of the virulent pneumococci, the quantities of fibrin present being only slightly larger than in the streptococcus exudates. The pneumonia produced by the avirulent pneumococcus differed further from the pneumonia of the virulent pneumococcus by the complete absence of the organism from the blood at any time after the inoculation. Even the pulmonary lesion itself contained no more culturable organisms on the second day after the inoculation.

By the non-fatal character of the disease, by the absence of bacteremia, by the scantiness of fibrin in the exudate, and by the tendency to earlier resolution, the pneumonia of the non-virulent pneumococcus manifests similarities with the experimental pneumonia produced by insufflation of the streptococcus. However, it differs from the latter by leaving the framework of the lung tissue and the bronchi practically free from injury. While in the advanced lesions caused by the streptococcus there was often a

marked purulent infiltration of the walls of the alveoli and of the bronchioles, of the connective tissue septa, and even of the adventitia of the blood vessels, in the pneumonic lesions brought on by the non-virulent pneumococcus there was at the most only a slight leucocytic infiltration of the walls of the small bronchi.

SUMMARY.

The intrabronchial insufflation of a non-virulent pneumococcus causes, like the insufflation of a virulent pneumococcus, the development of an exudate in the lungs which, in general, leaves the framework unaffected, and the lesion presents the gross appearance of a lobar pneumonia. It differs, however, materially from the pneumonia produced by virulent pneumococci in the important points that the consolidation tends to a more rapid resolution, the disease is non-fatal, the blood is not invaded by the organism, and the exudate is strikingly poor in fibrin.

As to the question which was the starting point for the foregoing investigation, namely, whether the pneumonic lesion produced by the streptococcus is merely a form of pneumonia caused by a less virulent organism, it may be answered for the dog, it seems, in the negative. The leucocytic infiltration of the framework of the lungs which occurs invariably in streptococcus pneumonia, and which is practically absent in the lesions caused by the virulent as well as by the non-virulent pneumococcus, is a strong enough feature to form a dividing line between the two forms of experimental pneumonia previously described.

AN EXPERIMENTAL STUDY OF BONE AND JOINT TUBERCULOSIS.*

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Much experimental work has been done with a view to demonstrating the origin and source of bone and joint tubercle, the paths by which the bacilli reach the bones, and the situations in which they develop.

Schüller (1) in 1880 described how he injected tuberculous material, sputum, glands, etc., through a tracheotomy opening into the lungs of dogs and rabbits, coincidentally injuring one of the knee joints of the infected animal; he succeeded in producing generalized tuberculosis and a tuberculous synovitis of the injured joint.

Müller (2) in 1886 injected the nutrient bone vessels of goats; apparently there resulted a multiple tuberculous osteomyelitis of the bone supplied by the infected blood vessel with accompanying infection of the neighboring joints. Müller quoted his results in demonstration of the hematogenous infection of osseous tubercle.

In 1891 Krause (3) published the results of his experimental work. He injected pure cultures of tubercle bacilli subcutaneously into guinea pigs and intravenously into rabbits. Directly before or immediately after the inoculation, or after a variable space of time, a joint was injured or a bone broken. In no case was there evidence of tubercle at the site of fracture, but in the instance of the joints many of them became tuberculous,—fifteen joints out of forty-four in guinea pigs, and fourteen out of twenty-eight in rabbits. The uninjured joints with one exception remained healthy.

A number of experiments upon rabbits were carried out by Benda (4) in 1899. He intimated his belief that the original lesion was an actual focus of tuberculous disease in the tunica interna of the blood vessel, and from such a focus there was a continuous liberation of bacilli into the blood stream. Lannelongue and Achard (5) (1899) found that it was by no means an easy matter to trace experimentally the source and origin of osseous or joint tubercle. They inoculated guinea pigs in various ways and directly afterwards or some time later they produced local injuries of the bones or joints, but they failed to produce tuberculous lesions.

With a view to investigating the hemic routes of infection, Friedrich (6) in 1899 introduced tuberculous cultures of low virulence into the left ventricle of rabbits. He succeeded in producing tuberculous joint affections. Certain

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of the joints had been previously injured, but he found that those subjected to traumatism were less likely to become infected than the apparently healthy ones.

What practically amounted to the antithesis of this view was expressed by Pietrzikowski (7) (1903). According to this view 20 per cent. of all tuberculous affections of bones and joints were connected with some forms of injury; this rarely amounted to a fracture or dislocation but usually an injury of lesser degree, such as a sprain or bruise.

In 1904 Salvia (8) injected virulent cultures of tubercle intravenously into rabbits; simultaneously various parts of the body were subjected to traumatism. He found that in the flat bones the violence practically always decided the localization of tubercle; no localization could be obtained in the long bones of the limb, but slight injuries to the parts sometimes resulted in tuberculous disease.

When one attempts to epitomize the experimental results, one is met by a mass of contradictions, but underlying these there are two facts which are clear: (1) that it is difficult to reproduce experimentally tuberculous lesions of the bones and joints; (2) that trauma as a localizing factor is slight in degree rather than severe.

In the course of an investigation into the etiology and pathology of bone and joint tubercle, a number of experiments were performed with a view to establishing (1) the routes by which infection spread to the locomotory system, and (2) the factors which governed the localization of the lesion. It is a brief outline of these experiments and their results which I wish to record.

GENERAL INFECTION EXPERIMENTS.

Local bone or joint tubercle is rarely the result of a generalized infection. To illustrate this fact a series of guinea pigs was inoculated with known quantities of human tubercle bacilli by two of the commonest routes of infection, subcutaneous inoculation and infection by feeding.

The first series, ten in number, was infected with amounts varying from one to two milligrams of dried bacilli. The inoculation was made subcutaneously, and six weeks later the animals were killed and examined. Special attention was paid to examination of the bones and joints, and in most instances the bones were submitted to microscopical examination. The post-mortem examination revealed in each case the presence of a disseminated tuberculosis. In no instance was any lesion discovered in the bones or joints.

Infection by ingestion was carried out by feeding four guinea pigs for a period of three weeks upon food adulterated with tubercle bacilli.

The animals were permitted to live for six weeks and they were then killed and examined. Post-mortem examination revealed the presence of general tuberculosis, apparently primarily beginning as a mesenteric infection. Examination of the bone and joints yielded entirely negative results.

These experiments illustrate the fact that infection of bones or joints is not a likely possibility in the general dissemination of tubercle.

LOCAL DIRECT INFECTION OF THE BONE.

A most important question to be enquired into is the result of a direct inoculation of the bone. The infection was carried on as follows:

Technique.—The tibia was usually chosen. The animal was anesthetised with ether and with aseptic precautions the bone was exposed from its inner or subcutaneous aspect. For about a third of the circumference the periosteum was separated from the underlying bone until an area of 1.9 cm. was exposed. In this area the medulla was opened by raising a tiny trap door of bone. This may be done with strong, sharply pointed scissors, but considerable care must be exercised to prevent fracture of the fragile shaft. The medulla must be inoculated without infection of the soft parts. The most satisfactory method of ensuring this was to introduce into the medullary cavity with a fine probe a tiny plug of cotton wool infected with tubercle bacilli. The wound was then bathed with antiseptic lotion, and the trap door of bone and the raised periosteum were replaced.

Guinea pigs and rabbits were used in the experiment and the human bacillus was employed. I shall first describe the results obtained by infection of guinea pigs. A series of seven animals was employed, and at the end of a varying period each animal was killed and examined. The infected bone was removed intact and submitted to microscopical examination. The more important details are expressed in table I.

Results.—The duration of time which each animal was allowed to live after inoculation varied from ten to sixty days. The post-mortem examination revealed in every case, as was to be expected, a general tuberculosis. The examination of the infected

bone showed that in four of the seven cases a definite tuberculous osteomyelitis had developed; but the negative results of the remaining three cases demand the chief attention, as microscopic examination afforded no sign of active tubercle; in one instance there existed what apparently was a healed tuberculous focus. In the negative cases one was convinced that there was no source of error in the original infection of the bone, but that they were instances of true recovery from infection.

TABLE I.

Local Inoculation of the Bone of the Guinea Pig.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	General examination.	Local examination.
A	Oct. 11	Oct. 20	10 days	Gland tubercle	Early T. B. Osteomyelitis.
B	Sept. 23	Oct. 17	20 days	General tubercle	T. B. Osteomyelitis.
C	Oct. 19	Nov. 22	30 days	General tubercle	Healed T. B. Osteomyelitis.
D	Oct. 4	Nov. 14	40 days	General tubercle	T. B. Osteomyelitis.
E	Oct. 2	Nov. 14	43 days	General tubercle	No tuberculous lesion.
F	Oct. 21	Dec. 10	50 days	General tubercle	No tuberculous lesion.
G	Oct. 8	Dec. 6	60 days	General tubercle	T. B. Osteomyelitis.

A similar series of experiments was performed upon rabbits. The rabbit possesses a considerable power of resistance to infection by the human tubercle bacillus, while it is unusually susceptible to inoculation with the bovine bacillus. In this series two rabbits were infected with a bacillus of human strain, while two were infected with a bovine bacillus. The details are given in table II.

TABLE II.

Local Inoculation of the Bone of the Rabbit.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	Type of bacillus.	General examination.	Local examination.
A	Nov. 10	Feb. 20	102 days	Human	Slight pulmonary T. B.	No active tubercle.
B	Nov. 10	Feb. 28	110 days	Human	Slight pulmonary T. B.	No T. B. Osteomyelitis.
C	Nov. 30	Jan. 10	40 days	Bovine	No general T. B.	Slight T. B. Osteomyelitis.
D	Nov. 30	Dec. 20	20 days	Bovine	No general T. B.	Slight T. B. Osteomyelitis.

Results.—When rabbit bones are directly infected with the human tubercle bacillus there results no local development of disease. When the bones are infected with the much more virulent bovine bacillus, the virulence of which had been previously demonstrated, a slight lesion appears, but it is circumscribed and retrogressive. It is interesting to note that the rabbits which were inoculated with bovine bacilli showed no evidence of general tuberculosis. The explanation apparently is that the cellular action is so intense as to prevent any general spread of the disease.

These experiments were carried out in fully developed animals and the center of the shaft was the site of local infection.

LOCAL INFECTION OF THE METAPHYSIS OF THE RABBIT.

In order to reproduce more exactly the type of infection which clinically occurs, a series of experiments was performed in which young rabbits with growing epiphyses were used, and the bones were infected at what is considered the site of election of the disease; namely, the metaphysis.

Technique.—The bone was opened at its most accessible part, usually the center of the diaphysis. Along the interior of the shaft, and with as slight damage to the marrow as possible, a fine capillary tube was passed until the progress of the tube was arrested at the growing epiphyseal extremity. To the tube so placed a piece of fine rubber tubing and a syringe filled with tubercle bacillary emulsion was attached, and along the tube a single drop of the emulsion was injected. In withdrawing the tube it is important to prevent as far as possible further infection of the medulla, and this can, to a certain extent, be done if while the tube is being removed a negative pressure is maintained by slightly withdrawing the piston of the syringe. The details of the experiment are given in table III.

TABLE III.

Local Infection of the Metaphysis.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	Type of bacillus.	General examination.	Local examination.
A	Dec. 10	Feb. 28	90 days	Human	Negative	Negative
B	Dec. 18	Feb. 18	62 days	Human	Negative	Negative

Results.—Both the above experiments yielded negative results. In neither case was there any development of the disease, local or general.

Conclusion.—The main fact illustrated by the above experiments is the remarkable difficulty which there is in infecting with tubercle the medulla of a healthy bone.

The guinea pig, the most susceptible of all animals to such an infection, shows a certain proportion of recoveries when the bone marrow is infected.

In the case of the rabbit its natural immunity to the human bacillus prevents entirely the development of a marrow lesion, and even when the bovine bacillus is inoculated the resulting disease is slight and retrogressive.

Evidence such as this suggests that clinically a primary infection of the bone medulla, a primary tuberculous osteomyelitis, is rare and unlikely.

DIRECT INFECTION OF JOINTS IN THE RABBIT.

The next question to consider is the result of direct infection of the joint cavity. With a view to elucidating this point a series of experiments was performed. Rabbits were chosen for the experiments and both human and bovine bacilli were employed in individual instances.

Technique.—The left knee joint was chosen, as being one of the most accessible both for inoculation and later observation. The interior of the joint can easily be entered if the knee is firmly flexed and the needle inserted immediately by the side of the ligamentum patellæ (table IV).

After the inoculation the animal was allowed to use the part as before.

TABLE IV.

Local Infection of Joints.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	Type of bacillus.	General examination.	Local examination.
A	Feb. 2	June 20	138 days	Human	Negative	Chronic T. B. Synovitis.
B	Feb. 7	June 15	128 days	Human	Negative	Chronic T. B. Synovitis.
C	Feb. 14	Mar. 30	44 days	Bovine	Slight pulmonary T. B.	Acute T. B. Synovitis.
D	Feb. 14	Mar. 30	44 days	Bovine	Pulmonary tubercle	Acute T. B. Synovitis.

Results.—In striking contrast to the bones, the joints are readily infected with the tubercle bacillus. As is to be expected, the

degree of reaction which occurs is much more intense in the case of the bovine bacillus than in that of the human.

The probability is that the liability of the joints to infection as compared to the bones depends upon the absence in the former of resistive cell elements. The drawback to experiments like the above lies in the fact that such massive direct infections can never actually occur *in vivo*. In order to reproduce a typical clinical infection it is necessary to secure a more general and gradual dissemination and with this in view a further series of experiments was performed.

GENERAL INFECTION VIA THE MESENTERIC VESSELS IN THE RABBIT.

Children, the main sufferers from bone and joint tubercle, are infected in the majority of instances by the ingestion of tuberculous milk (Fraser (9), 1912); the primary focus lies in the mesenteric lymph glands, and from such a deposit, dissemination occurs.

To reproduce this type of infection a series of experiments was performed in which a quantity of tubercle bacilli was introduced into the circulation by injection of a mesenteric vein.

Technique.—Rabbits were employed in the experiments. The animals were anesthetised with ether and the abdomen was shaved and sterilized. An incision was made in the middle line and a loop of small intestines extracted. With a fine hypodermic syringe and needle one of the larger mesenteric veins was punctured and a quantity of human bacillary emulsion slowly injected. After withdrawing the needle the bleeding was sometimes so severe as to necessitate ligation of the punctured vessel. The abdominal wall was finally closed (table V).

TABLE V.

General Infection via the Mesenteric Veins.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	Result of experiment.
A	Dec. 7	Feb. 4	59 days	Tubercle of liver and peritoneum; bones and joints healthy.
B	Dec. 7	Dec. 22	15 days	Tubercle of liver and lungs; bones and joints healthy.
C	Dec. 15	Dec. 30	15 days	Tubercle of liver and lungs; bones and joints healthy.
D	Dec. 16	Jan. 14	30 days	Tubercle of liver and lungs; bones and joints healthy.

Results.—Uniformly the results have been negative. Infection of the mesenteric veins necessarily produced tuberculous foci in the

liver and lungs and occasionally in the overlying peritoneum, but in no instance was there any sign of infection of the bones or joints.

GENERAL INFECTION BY DIRECT INOCULATION OF THE HEART BLOOD.

It has been suggested that the incidence of bone tubercle owes its origin to a hematogenic infection, and a series of experiments was performed to demonstrate the possibility of its occurrence.

Technique.—I have previously alluded to the experiments of Friedrich in this relation. Friedrich infected the blood stream by passing a tube into the left ventricle along the carotid. The method which I adopted was somewhat different and simpler in execution. It is possible to enter the left ventricle by inserting a needle through the chest wall. Under an anesthetic this was done and the emulsion was slowly injected. It is rapidly disseminated by the arterial blood throughout the body. Of course the most obvious way to infect the circulation is by intravenous injection, but this leads to such a massive infection of the lungs that death results very early. It is by no means difficult to enter the ventricle through the chest wall and I have demonstrated by post-mortem dissection the certainty of the method (table VI).

TABLE VI.

General Infection via the Left Ventricle.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	General examination.	Local examination.
A	Oct. 29	Mar. 3	125 days	Pulmonary tubercle	Bones and joints healthy.
B	Nov. 7	Mar. 3	116 days	Pulmonary tubercle	Bones and joints healthy.
C	Nov. 12	Mar. 20	128 days	Pulmonary tubercle	Healed tubercle center right tibia.
D	Dec. 9	Mar. 3	84 days	Pulmonary tubercle	Bones and joints healthy.
E	Dec. 16	Jan. 7	22 days	Pulmonary tubercle	Bones and joints healthy.
F	Dec. 16	Jan. 8	23 days	Pulmonary tubercle	Bones and joints healthy.

Results.—In a total of six experiments a local infection developed in one instance, and this single positive result was indicated not by an active tubercle, but by a healing and retrogressive process. The value of these results lies in their demonstrating that a circulation of tubercle, a tuberculous septicemia, in fact, unaided by any predisposing factors, is unlikely in itself to produce a local tuberculous lesion of the bone or joint.

DIRECT INFECTION OF THE MAIN VESSEL SUPPLYING A LIMB OF THE RABBIT.

While a general arterial infection may yield negative results, it seemed possible that a more direct infection of the vessel supplying a limb might give more promising results.

Technique.—Rabbits were employed in the experiments. The femoral vessels were exposed upon the left side immediately beneath the groin, and the sheath of the vessel was opened and the artery separated for a distance of about 1.27 cm. For the purpose of injecting the vessel a specially prepared needle was employed, bent to a right angle about 1.9 cm. from its tip. The vessel lumen was entered with the needle and gradually about 0.5 c.c. of tubercle emulsion was slowly injected in the direction of the blood stream. The injected fluid was thoroughly distributed by a few pulsations of the heart. The bleeding subsequent to withdrawal of the needle was so severe as to necessitate in almost every case ligature of the punctured vessel. The wound was closed with sutures, and the animal allowed to use the limbs as before (table VII).

TABLE VII.

Direct Infection of the Main Blood Vessel of the Limb.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	General examination.	Local examination.
A	Oct. 19	Mar. 3	135 days	Pulmonary tubercle	Bones and joints healthy.
B	Oct. 26	Jan. 5	63 days	Pulmonary tubercle	Tuberculous disease of left ankle.
C	Oct. 29	Mar. 3	127 days	Pulmonary tubercle	Tuberculous metatarsophalangeal joint.
D	Nov. 7	Mar. 3	117 days	Pulmonary tubercle	Bones and joints healthy.

Results.—These experiments show that it is possible to infect the joints of a limb by infection of the main blood vessel with tubercle.

The primary infection was of the joint, and any change which may have appeared in the neighboring bone was secondary to the synovial tubercle. This was demonstrated by subsequent microscopic examination. The infection had originated in that portion of the synovial membrane which lines more especially the reflection of the joint.

Histologically the changes in the joint subsequent to infection were those of an acute synovial tubercle.

DIRECT INFECTION OF THE NUTRIENT ARTERY OF THE RABBIT.

While infection of the main blood vessel leads to tubercle of the joints of that limb, the question arose as to the result of direct infection of the nutrient artery, the injection being so directed as to enter entirely the medulla of the bone.

Technique.—The methods employed were similar to those used by Cheyne (10).

The experiments were performed upon rabbits. The nutrient artery supplying the interior of the tibia springs from the femoral artery, a short distance below the knee, and enters the tibia obliquely from above downwards, at the junction of its upper and middle thirds.

The nutrient vessel is much too fine to permit of direct inoculation, and therefore one has, so to speak, to procure its infection indirectly. The femoral artery is tied a short distance below the level at which the nutrient vessel is given off, the artery is exposed higher up just below the groin, and there inoculated with fine needle and syringe. The blood stream must therefore follow the only path which remains open to it, namely the nutrient vessel, and it carries with it into the interior of the bone the infected material.

TABLE VIII.

Direct Inoculation of the Nutrient Artery.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	General examination.	Local examination.
A	April 2	April 20	18 days	Pulmonary tubercle	Tibia healthy.
B	April 5	Sept. 7	5 months	Pulmonary tubercle	Tibia healthy.

Results.—In both cases the result of the local infection was negative. There was, of course, some evidence of general infection, but the infected tibia, when examined microscopically, showed a fatty change in the marrow, but no evidence of the development of tuberculous tissue.

GENERAL CONCLUSIONS.

While one can not assert that the conclusions which experiments appear to offer must of necessity correspond to the conditions which arise clinically in man, nevertheless these results, if definite, must tend to show the direction in which the truth lies.

The conclusions which may be drawn from this research are as follows:

1. Direct infection of the medulla of a long bone is unlikely to lead to the development of a tuberculous osteomyelitis.

2. Inoculation of the interior of a joint with tubercle bacilli readily produces tubercle of the synovial membrane.

3. From such an infected joint the epiphysis or metaphysis of the bone becomes diseased.

4. Infection of the arterial heart blood does not result in the local development of tubercle of the bones or joints.

5. Infection of the main artery supplying a limb leads to the development of tuberculous disease of certain of the joints of that limb.

6. Direct infection of the nutrient artery does not result in tuberculous osteomyelitis of the bone.

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